The Impact of Oil Palm Conversion on Tropical Amphibians

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Declaration

I certify that this thesis, and the research to which it refers, are the product of my own work, and that any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline. I acknowledge the helpful guidance and support of my supervisors, Dr Trenton W. J. Garner and Dr Robert J. Knell.

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The world's mine oyster, Which I with sword will open
-William Shakespeare

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Abstract

Agriculturally-altered habitats, especially oil palm plantations, are rapidly dominating the Southeast Asian landscape. Although recent studies have shown reduced species diversity associated with this commodity, data on amphibian diversity are rare. The following thesis explores the impact of oil palm plantations on amphibians in Peninsular Malaysia based on (1) amphibian biodiversity, (2) quality and use of breeding sites, (3) habitat use and (4) parasitism. Contrary to expectation, not all metrics of biodiversity differed between oil palm plantations and secondary forest sites. Amphibian community composition, however, differed greatly between the two habitat types, with oil palm communities being dominated by species known to prosper in disturbed habitats, indicating that the community is currently of limited conservation value. Within plantations, temporary pools were found to serve as important breeding habitats for amphibians so a focused study on the characteristics of these pools was carried out. Although we found differences between pools, the proportion of occupied pools did not differ significantly between plantation and forest sites. I did observe evidence of breeding site preferences of least concern, plantation amphibians, along with habitat partitioning between species, a similar pattern also seen in forest communities. I compared parasite burdens between habitats by screening for the fungus *Batrachochytridium dendrobatidis* (*Bd*) and for nematode parasite load. *Bd* was not detected in any of my samples and there was no difference in nematode loads between habitat types. However, patterns of nematode prevalence was affected by host type, while nematode intensity was dependent on an interaction between host and body size. The final chapter indicates that in terms of parasite, the host environment is the most important. Additionally, differences in host-parasite patterns between habitats indicate a possible

underlying problem that rapid biodiversity censuses would be unable to detect.

Chapter 1

Introduction

1.1 Introduction

This chapter will provide an overall introduction to the thesis. It begins with an introduction to oil palm agriculture in Southeast Asia, specifically focusing on Malaysia. This is then followed by a discussion of the positive and negative aspects of this commercial crop. I will outline the gap within the knowledge base and the reasoning for studying amphibians. Finally, I will end with the aims, the possible limitations and the organization of the thesis.

The impact of a rapidly growing human population on natural habitats is becoming increasingly difficult to ignore. With a two fold increase over the past four decades to about 6.5 billion people in the world and a projected increase to around 9 billion by 2050 (Population Reference Bureau, 2006), such expansions would inevitably be coupled with increasing demands for food and space. From 1971 to 2010, the global consumption of edible vegetable oil has increased from 16 million tons to 115 million tons, with China, India, the United States and the European Union being the top four consumers of vegetable oils (Koh & Lee, 2012). The major source of vegetable oil and fat is currently derived from oil palm (*Elaeis guineensis*), the majority of which comes from the tropics (Koh & Lee, 2012). The area used for oil palm production has increased dramatically and now covers 10.7 million ha worldwide (Basiron, 2007).

Southeast Asian countries, specifically, Malaysia and Indonesia, are the main players in oil palm production, and agricultural expansion within these areas have exhibited exceptional growth from 3 million ha in 1971

to over 9 million ha in 2009 (Gallant *et al.*, 2007; Gibbs *et al.*, 2010). Currently, both these countries collectively produce more than 80% of the worlds palm oil (Fitzherbert *et al.*, 2008; Koh, 2007), which is used in domestic cooking, commercial food products, feed for livestocks and recently, its possibilities as biofuels (Basiron, 2007; Turner & Foster, 2008; Fitzherbert *et al.*, 2008). Rapid development of the palm oil industry in countries like Malaysia is mainly attributed to the crops high yields per unit area (∼30 years of productive life), strong global demand for fats and oils and favourable climatic conditions, which makes the crop easily adaptable from its origin of West Africa (Donald, 2004; FAO, 1998). Additionally, rising labour costs, decreasing demands for natural rubber and the plight of cocoa borer pest have further increased the incentive for planting oil palm over these previously lucrative commercial crops (FAO, 1998). In Malaysia, a series of economic policy decisions, one of which is increasing exports of commodities such as oil palm have helped boost its economy. As a result, Malaysia's Gross Domestic Product [GDP] (the total expenditures for goods and services produced within the country) has risen from $4.5 billion in 1972 to $278.67 billion in 2011, making it one the worlds economic success stories. In 2009, the poverty head count in Malaysia decreased to only 3.8% of the population, with 99% of the population having access to safe drinking water, health care and education facilities (World Bank, 2012).

Due to the increasing demands for oils and fats, along with its advantages to the economy of developing countries, oil palm production in the future is thought to double (Fitzherbert *et al.*, 2008). Increase in production would go hand in hand with the expansion of oil palm cultivation, which is cited as being a major factor in driving deforestation and loss of local diversity (Turner & Foster, 2008). In South East Asia, tropical forests hold more species compared to temperate forests, have the most number of endangered species and store 46% of living terrestrial carbon (Danielsen *et al.*, 2009). Malaysia and Indonesia possess more than 80% of South East Asia's remaining primary forests (Fitzherbert *et al.*, 2008) and palm oil production is thought to threaten the endemic diversity in these areas (Aratrakorn *et al.*, 2006). In Malaysia alone, 89 species of

amphibians do not occur anywhere else in the world and 47 of them are listed in the IUCN Red List as threatened. In Indonesia, 146 species of mammals are also listed as threatened by the IUCN (Koh, 2007). A study of Malaysias landscape revealed that the major cause of forest fragmentation in Peninsular Malaysia was due to agricultural oil palm and rubber plantations, where oil palm was specifically associated with fragmentation of wetland landscapes (Abdullah & Nakagoshi, 2007).

Despite being one of the most widely exported and consumed vegetable oil globally, research interest on oil palm, compared to other oil crops, is grossly limited. Based on publication searches done by Turner and his colleagues (2008), most of the research surrounding oil palm were related to increasing yield, alternative usage and health issues, with less than 4% focusing on its impact on biodiversity at the time. Publications investigating the effects of oil palm cultivation on biodiversity consistently showed plantations having a lowered diversity, abundance and a community commonly found inhabiting disturbed habitats (Donald, 2004; Aratrakorn *et al.*, 2006; Koh & Wilcove, 2008; Fayle *et al.*, 2010; Gillespie *et al.*, 2012). Conversion to plantations from primary forests resulted in a decrease of bird species richness up to 77% (Aratrakorn *et al.*, 2006). Fayle and colleagues (2010) found that primary forests supported three times the amount of ant species compared to oil palm monocultures, while the number of bat species decreased more than 75%, with a change in the species composition by 60% (Donald, 2004; Struebig *et al.*, 2009).

Converting any natural habitat into a monoculture was always going to have an effect on the native ecosystem, but measuring such effects can be tricky, as not all organism responds the same way. Changing the structure of a habitat could have a negative relationship with species diversity, as seen in the community of birds, ants and bats previously studied in oil palm plantations. However, not all species show a reduction in numbers. The density of wild pigs (*Sus scrofula*) in disturbed habitats such as forest fragments and oil palm plantations was seen to increase (Ickes, 2001). Similarly, the increase in blood pythons (*Python brongersmai*), short-tailed pythons (*P. curtus*) and the Malaysian field rat (*Rattus tiomanicus*) were also reported following conversion (Wood & Liau, 1984; Shine *et al.*, 1999).

There is also the possibility that within a class of organism, the amount of winners could be as many as the amount of losers, resulting in no changes in species richness, but considerable change in communities (Devictor *et al.*, 2009). If the case remained that oil palm plantations are unable to support high biodiversity, the question is whether managing plantations with increasing biodiversity in mind, is actually possible? Indeed, Koh and colleagues have shown that the inclusion of natural forest cover within and around plantations positively influenced the number of forest bird and butterfly species. With the rising public awareness of the negative effects of oil palm, pressure was put onto the industry to ensure environmentally friendly and sustainable production. A number of groups, the most influential of those being the Roundtable on Sustainable Palm Oil (RSPO), were formed over the last few years with the aim of investigating, maintaining and promoting the sustainability of palm oil cultivation (Turner & Foster, 2008; RSPO, 2012). However, if much progress is warranted on ensuring palm oil as environmentally friendly and sustainable, the current knowledge base of the impact of this commodity is still insufficient (Turner & Foster, 2008; Bakewell *et al.*, 2012). For example, basic biodiversity surveys of a number of animal taxa are still lacking, along with our understanding of its effects on plant-pollinator, predator-prey and/or host-parasite relationships (Bakewell *et al.*, 2012).

One of the lesser-studied taxa within this topic are amphibians. Globally, amphibians are one of the most threatened groups of vertebrates (Cushman, 2006). The conclusions from 118 monitoring programmes are that there are widespread population declines affecting around 30 genera and nine families within the class (Gardner, 2001). In addition to this, the IUCN Global Amphibian Assessment has reported that 32% of over 6000 known amphibian species in the world are under threat from extinction (Greenwood & Robinson, 2006). Abnormally high rates of amphibian declines were first discussed at the First World Herpetology Conference in 1989 and scientifically reliable observations were made during the 1980s till the early 1990s (Gardner, 2001). Amphibians possess certain characteristics, which make them susceptible to changes in the environment (Guerry & Hunter-Junior, 2002). Having a permeable skin and being ectothermic

means that amphibians depend on their external environment for temperature and moisture regulation (Gallant *et al.*, 2007). This characteristic limits the size of home ranges and ties populations to moist microclimates (Cushman, 2006; Guerry & Hunter-Junior, 2002). Agricultural land conversions and logging can change the structure and climatic characteristics of a particular habitat, which can be detrimental to local amphibian species (Vallan, 2002; Krishnamurthy, 2003; Gardner *et al.*, 2007a). Along with being affected by habitat alteration/loss, parasite and infectious disease have been central to threats on amphibian populations. Pathogen pollution or the introduction of pathogens into new areas is an increasing threat to global biodiversity and in 2001, the National Research Council listed it as one of the eight most urgent environmental issues (Johnson & Chase, 2004). This phenomenon has been greatly increased due to the increased mobility of humans, which leads to the translocation of wildlife, soil, ballast water and plants (Daszak *et al.*, 1999). Furthermore, the parasite prevalence, intensity and distribution can be affected by the overall habitat, a topic area that has had little attention over the years despite a rapidly changing world (Koprivnikar *et al.*, 2012).

Global distribution of amphibians show a higher number of species found in tropical Asia compared to tropical North America (Duellman, 1999). In the Old World tropics, the most specious families include Ranidae followed by Microhylidae, Rhacophoridae, Hyperolidae, Hylidae and Myobatrachidae, with a combined number of $\sim$1, 767 known and described species (Hero *et al.*, 2003). Although there is mounting evidence for the decline of amphibian populations around the world, there are certain areas, particularly around South East Asia, where even the species list of amphibians have not been fully compiled. There is a skewed distribution of amphibian researchers, with a higher proportion found in North America, Australia and Europe (Gardner, 2001). Such distributions have led to a research bias on the knowledge of certain species ecological preferences. This is extremely unfortunate, as the species richness of amphibians tend to be highest in humid ecoregions such as those found near the equator (Gallant *et al.*, 2007). Amphibians are known to have an impact on both natural ecosystems and human welfare (Gardner, 2001; Vallan, 2002).

Due to their complex life-cycle, most amphibians will have an effect on both the terrestrial and aquatic environment during their lifetime (Vallan, 2002). They are thought to be an essential part of many ecosystems, contributing significantly to the vertebrate biomass (Gardner, 2001; Vallan, 2002). In both temperate and tropical ecosystems, amphibians play a crucial role in trophic dynamics, acting as both the predator and prey species (Gardner, 2001; Vallan, 2002); maintaining invertebrate abundance and providing nourishment for higher order predators (Gardner, 2001). Thus, their decline could potentially have a detrimental effect on ecosystems, especially in the tropics, where amphibian biodiversity is exceptionally high. To date, there has been only one other study looking into the impact of oil palm on amphibian diversity (Gillespie *et al.*, 2012). The presence of such studies helps to improve our understanding of habitat alteration and amphibians in Southeast Asia, however, it only provides a basic and broad comparison between habitat types. Although general comparisons are indeed, valuable, this thesis will highlight how, such methodologies can be misleading, and in terms of amphibians in Malaysia, cannot be generalized across the country. In addition, more detailed information is needed above species richness and composition if we are to improve on the way we manage habitats across Malaysia.

1.1.1 Thesis aims

The aim of this research project has therefore been to try and increase the knowledge base surrounding oil palm plantation and amphibians in Malaysia. By identifying if the negative relationship between this type of commodity and amphibian diversity is consistent with all other biodiversity studies done in the past decade. To investigate the variables associated with conversion at different levels; forest versus plantation, stream versus terrestrial and finally, the location of species A versus the location of species B. Additionally, to study the typical behaviour of species found in this type of altered habitat and identify their breeding requirements. Finally, to touch on host-parasite patterns and investigate the differences between two habitat types.

1.1.2 Synopsis of the method and possible limitations

Four works will be examined, data of which were collected from two secondary forest sites and three plantation sites. It is beyond the scope of this study to provide primary forest data in this study. The majority of Malaysia's forest is not primary and a considerable amount of secondary forests are likely candidates for conversion into oil palm plantations (FAO, 1998; Koh & Wilcove, 2008; Gillespie *et al.*, 2012). Even though secondary forests may not support as many species as primary forests, such habitats are still more complex compared to monocultures with a higher amount of species (Aratrakorn *et al.*, 2006; Fitzherbert *et al.*, 2008; Gardner *et al.*, 2007b; Vallan, 2002; Edwards *et al.*, 2010). Undisturbed or primary forests that remain in Malaysia are typically at high latitudes, on karst outcrops or already protected as forest reserves. These would be unsuitable for plantation conversion (Struebig *et al.*, 2009), and for certain isolated outcrops, generally unsuitable for amphibians (Gillespie *et al.*, 2012). In addition to this, the European Commission has plans to ban the import of fuel crops that are planted on certain landscapes, including primary tropical forests (Koh & Wilcove, 2008).

Due to practical constraints, temporal comparisons could not be made (i.e.before and after conversion). Newly converted plantations are rare within Peninsular Malaysia as the area available for conversion has reached saturation (Koh & Wilcove, 2008). Plans for expansion within Malaysia is typically restricted to Sabah and Sarawak, of which large experimental studies are already underway. I chose to focus on plantations erected before any sustainability schemes were put in place, as these plantations are still within areas of high biodiversity and should not be left unmanaged. Furthermore, these plantations will still need the backing of NGOs and programmes like RSPO in order to maintain its trading with international companies and alleviate pressures from environmental bodies. By studying the patterns of mature plantations, we will be able to figure out ways of improving these plantations for increased amphibian diversity.

Another potential problem is the lack of detail in the parasitic nematode data as taxonomic identification could only be done to genus level.

Nematode species are notoriously difficult to identify at the best of times, and males are typically needed for this process to even occur. Males, however, were extremely rare in our sample, therefore, the females were used to identify the genus found in each host.

1.1.3 Thesis organisation

The overall structure of this study is assembled into six chapters: four data chapters, one introductory chapter and finally, the overall conclusions. The data chapters, i.e. the meat of this thesis, takes the form of typical journal papers. The reasoning for composing these chapters as such is mainly to make it easier for the reader to follow the themes and aims outlined for each chapter. Chapter 2 is basically a platform for the whole thesis as it introduces the study area and general survey methodology. In the same chapter I investigate the impact of this commodity on amphibian diversity, abundance and community composition. I compare each biodiversity metric among habitat types and also among different macrohabitats (stream, riparian and terrestrial). The chapter also emphasizes the importance of using appropriate methodology when trying to identify responses to habitat alteration in monitoring studies. The rest of the data chapters follow on from the results of Chapter 2. Chapter 3 aims to test the disturbance-generalist paradigm of amphibian species in plantation sites, specifically in the context of breeding site choice of temporary pool breeders. Here, I initially compare the structure and proportion of pools used, along with identifying tadpole feeding guilds among habitats. Chapter 4 compares structural heterogeneity between habitat types. I also evaluate microhabitat associations and partitioning of abundant species found in plantation compared to those in forest sites. The final data chapter, Chapter 5, takes a rather different turn, as I go into the world of host-parasite patterns. The overall theme is the same; evaluating the effects of habitat alteration, however, here, I compare parasite prevalence, intensity, frequency distribution and diversity between amphibian hosts found in the two habitats. I focused on both micro- and macroparasites

known to infect amphibians. The final chapter draws upon the entire thesis, tying up the various results and conclusions made throughout the four data chapters with possible directions for future research and applications towards plantation management.

Chapter 2

The impact of oil palm plantations on species diversity of tropical amphibians

2.1 Introduction

Biodiversity loss is most commonly attributed to habitat loss and alteration (Stuart *et al.*, 2004; Todd & Rothermel, 2006). The rise in global demand for agricultural commodities is a major driver of habitat modification, and the amount of land committed to cropland is predicted to expand substantially in the next four decades (Wanger *et al.*, 2009). A large component of this involves conversion to oil palm plantations, much of which has occurred in Southeast Asia (Koh, 2007). Oil palm has come to dominate this landscape, predominantly in Malaysia and Indonesia, and has steadily replaced other crops in these two countries due to its low maintenance requirements and high yield per unit area (Donald, 2004; Koh & Wilcove, 2008; Danielsen *et al.*, 2009). Suggestions have been put forward to discourage the planting of oil palm on primary and secondary forests and restrict it to cleared grasslands and other agricultural fields (Koh & Wilcove, 2008). Although large areas of old growth or low level disturbance forests have been gazetted and should be protected from large scale conversions, smaller areas of secondary forest remain unprotected and possibly under threat from future conversions. Koh and Wilcove (2008) reported that between 1990-2005, 55%-59% of oil palm expansion was at the expense of secondary forest. Secondary forests in Malaysia,

while not as diverse as old growth forests, still retain significant biodiversity and may contain unique assemblages when compared to monoculture plantations (Chazdon *et al.*, 2009).

Oil palm has been consistently associated with reductions in species richness, species diversity and shifts in community composition (beetles: Chung *et al.* 2000; reptiles: Glor *et al.* 2001; birds: Koh & Wilcove 2008; ants: Brühl & Eltz 2010) but to date there has been one study examining amphibian communities in oil palm plantations in Borneo (Gillespie *et al.*, 2012). The World Conservation Union (IUCN) Red List reports 41% of 6300+ described, extant amphibian species are at increased risk of extinction (IUCN: www.iucn.org), making the Amphibia the most threatened vertebrate class assessed to date. Pollution, over-harvesting and infectious diseases are all known contributors to the global amphibian decline, but habitat alteration consistently ranks as the most important threatening process. Southeast Asia is classified as an amphibian biodiversity hotspot, yet amphibian studies in this region are rare (Sodhi *et al.*, 2007, 2008). With 82% of global amphibian species largely forest-dependent, a pattern reflected in Southeast Asia, even slight alterations of forested landscapes have the potential to affect a large proportion of tropical amphibians (Ernst & Rödel, 2005).

The effects of habitat loss and alteration on amphibian biodiversity have been documented elsewhere, and amphibian responses are inconsistent (Hecnar & M'Closkey, 1997; Pearman, 1997; Gibbs, 1998; Parris, 2004). Alteration can make habitats unsuitable for amphibian reproduction and survival, as described in Australia, where conversion of *Eucalyptus* forest to pine monoculture reduced the number of detected frog species fourfold (Parris, 2004). However, in some cases altered habitats may still support a significant subset of the original amphibian community and retained species can utilize both pre- and post-conversion habitats (Gibbs, 1998). Amphibian species that are able to tolerate disturbed environments often have flexible breeding habitat requirements and high fecundity (Williams & Hero, 1998), so the effect of conversion on abundance, but not richness, has the potential to be reduced over time.

When habitat is rendered unsuitable for the original community, it

may still be colonized by previously rare or undetected species that have the ability to utilize the altered landscape. A decline in species richness and abundance evident soon after conversion may eventually be masked, given sufficient time and availability of species that can exploit the new, disturbed habitat. However, species diversity should exhibit strong correlations with habitat variables in both landscapes. This pattern has been observed in North America where the introduction of fish led to the loss of smaller, palatable amphibians native to the site and colonisation by larger, unpalatable amphibians (Hecnar & M'Closkey, 1997). Last, habitat alteration may result in some combination of the second and third outcomes, where a subset of the existing amphibian community persists in the altered habitat but new species colonize newly created niche space. Given time, little or no reduction in species richness or abundance would be detected, again due to colonization by disturbance-tolerant species. However species correlations with altered habitat variables would be inconsistent.

In this study I used a combination of species detection surveys, abundance counts and associated analytical approaches to describe abundance, species richness and community composition of amphibians sampled in oil palm plantations (established $>$ 30 years ago) and secondary forest (previously logged $>$ 50 years ago, intact forest with low level tribal hunting and extractive disturbance) located on peninsular Malaysia. By utilizing multiple methods, I avoided dependence on single indices, which cannot distinguish between the range of potential responses that an amphibian community may have to forest conversion. Based on previous studies of the effects of agricultural landscapes on amphibian biodiversity (Gardner *et al.*, 2007b; Wanger *et al.*, 2009), I predicted that oil palm will support decreased amphibian diversity, and be dominated by an amphibian community better suited to thrive in the disturbed monoculture that oil palm represents. Our results clarify what methodologies are best for comparing amphibian populations in oil palm monitoring programmes and provide guidance for the development of oil palm mitigation schemes.

2.2 Material and methods

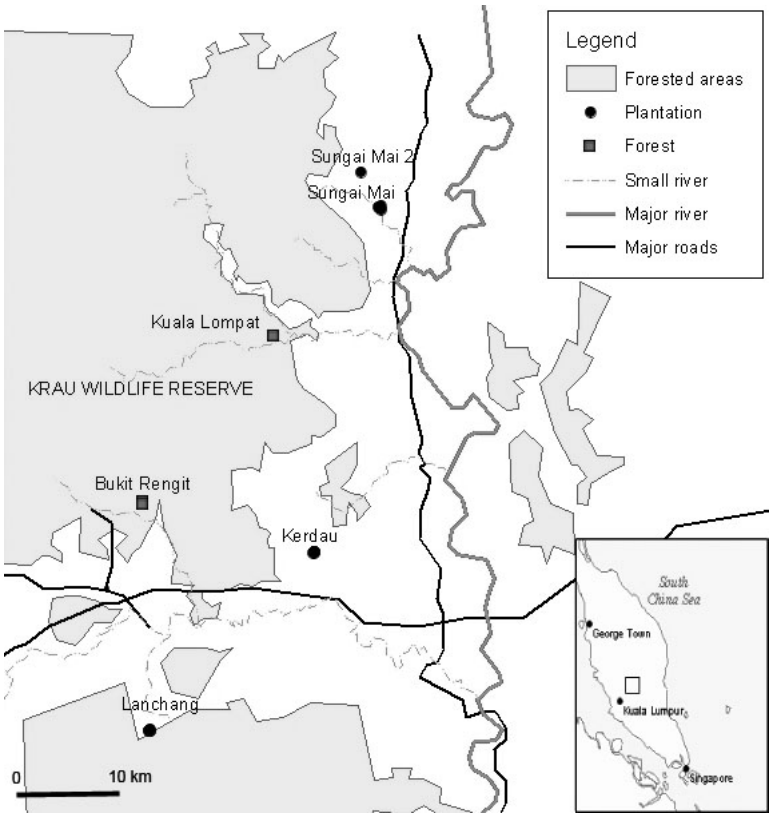
Study sites and field methodology

The study was conducted in the district of Temerloh within the state of Pahang, Peninsular Malaysia (3°40'N, 102°10'E). This state has the largest remaining forest cover, forest reserves and protected area within Peninsular Malaysia (Perhilitan, 2001). However, deforested landscapes due to increased urbanisation and the expansion of oil palm and other plantations now covers 43% of the state (Struebig *et al.*, 2009, Fig. 2.1). Two periods of maximum precipitation occur between September and December and between March and May, and temperature is also highly seasonal (Struebig *et al.*, 2009). Because temperature and rainfall patterns affect amphibian activity and detectability (Pellet & Schmidt, 2005) I surveyed at the same time each year, after the spring wet season. I also collected data on rainfall and temperature to confirm their consistency. I used rain gauges to measure rainfall and hand-held thermometers to measure night time temperature each night before I surveyed plots for amphibians.

I sampled a total of 57 plots (30m X 2m) (stream, riparian and terrestrial) split unevenly among two forest and four plantation sites (Fig. 2.2). I marked one stream at each site with flagging tape every 10m up to 400m or until the stream became dry. Start points for 30m stream survey plots were selected randomly from among the 10m lengths. Stream plots included 1 m of stream bank and the entire width of the stream. Start points for the riparian and terrestrial survey plots were also randomly selected from the 10m lengths marked on the stream, positioned perpendicular and running parallel to the direction of stream flow, but not overlapping with the stream plots or each other. Riparian plots were 2m wide and started 1m from the stream, while terrestrial plots were of the same width but located a minimum of 10m from the stream. All sites were visited weekly in a random order to standardize the effect of temporal variability of amphibian presence and density on analyses.

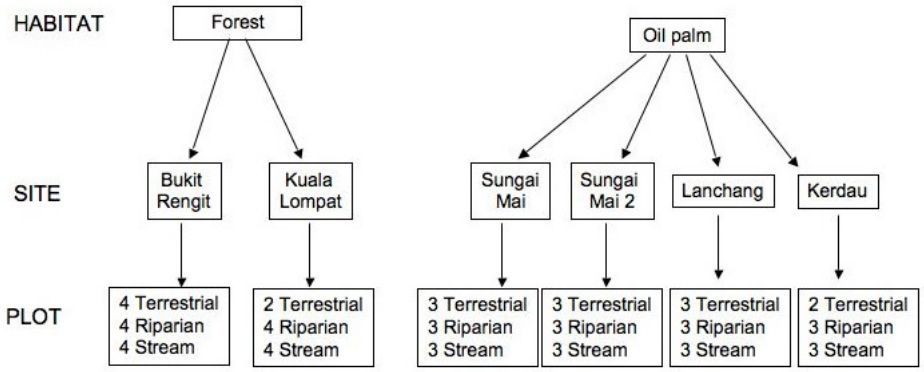
Each plot was actively searched for amphibians 7 times between May and July in 2009 (2009: total time spent on survey = 3568 minutes), and

Figure 2.1: Location of study sites in the Temerloh district. The insert is a map of Peninsular Malaysia with the location of Pahang boxed. Shaded areas represent forested areas and white areas consist of a plantation mosaic (oil palm, rubber, fruit and *Acacia spp.*), according to the Malaysian Ministry of Agriculture maps. Squares represent survey sites within the forested area. Solid circles represent survey sites within plantations.



4 times between May and June in 2010 (2010: total survey = 2195 minutes). I used direct visual searches to find stream dwelling and arboreal amphibian species but to find amphibians in riparian and terrestrial plots I used visual and disturbance searches and displaced leaf litter, logs and rocks. When possible, individual frogs were captured and photographed for later confirmation of species identification. Each individual was released at point of capture at the end of each plot survey. Any that were not captured but were clearly identifiable to species were included in the data set.

Figure 2.2: A schematic representation of the sampling design. Forest and oil palm are 'habitat type'. Bukit Rengit, Kuala Lompat, Sungai Mai, Sungai Mai2, Kerdau and Lanchang are 'sites'. Terrestrial, riparian and stream are 'plots'.



Prior to each plot survey I collected a series of macrohabitat measurements. Care was taken to ensure sampling for environmental variables did not disturb amphibians either into or out of survey plots and I did not observe any evidence of such short-term movement associated with the collection of environmental variables. I estimated percent canopy cover, soil pH, percent leaf litter cover, leaf litter depth and weight, percent undergrowth vegetation cover, number of trees (<50cm and $\geq$ 50cm diameter at breast height) and logs (<50cm and $\geq$ 50cm maximum diameter) for all riparian and terrestrial plots. I estimated canopy cover from digital photographs of the canopy taken with a Nikon D40 SLR camera at the beginning, middle and end of the plot (Nikon 10.5mm f/2.8 DX fisheye lens) using ImageJ (Rasband, 2011). I set a minimum threshold value of 100 and pixels that exceeded this value were taken to represent canopy cover (black pixels). I averaged the percentage of black pixels across the three points to estimate canopy cover for each plot. Similarly, I measured soil pH, estimated leaf litter characteristics and percent undergrowth vegetation using the same 3 point sampling system (beginning, middle and end) within each plot and averaged measurements per plot. Leaf litter cover was estimated in 1m X 1m quadrats, while undergrowth vegetation

cover was estimated in 2m X 2m quadrats. Both were estimated visually and I recorded what percent each variable covered within the 1m X 1m and 2m X 2m quadrat. I estimated leaf litter depth 3 times at each of the 3 points. Leaf litter from every 1m x 1m quadrat was collected and its wet weight measured in the field. Macrohabitat measurements were repeated for each sampling year.

Within each stream plot, I estimated the mean width (average of maximum and minimum length) and maximum depth of the wetted channel. Density of vegetation up to 1m from the wetted channel was estimated based on the method proposed by Keller *et al.* (2009), where the apex of individual plants was categorized into one of three height categories (0m-10m, 11m-100m and 101m-200m). The same 3 point sampling system was used from the riparian and terrestrial plot survey to obtain water pH, dissolved oxygen (DO) content, conductivity and total dissolved solids (TDS) using a multivariable-YSI kit (YSI Incorporated, USA). Percent of each substrate type was estimated using a 2m X 2m plot using the same 3 point sampling system. Apart from detritus (dead, floating vegetation) and sand, substrate types were assumed to be rounded in shape and classified based on their estimated diameters: gravel ($\geq$ 10mm, <30mm), pebbles ($\geq$ 30mm, <60mm), cobble ($\geq$ 60mm, <200mm) and boulders ($\geq$ 200mm).

Data analysis

The average nightly temperature and weekly rainfall were compared between habitat types and per year using a Pairwise Wilcoxon test. I identified differences in frog abundance between habitat and plot types using generalized linear models (GLMs) with Poisson errors in R statistical software (R Developmental Team, 2012). I detected over-dispersion while fitting the Poisson GLM of the abundance data and subsequently corrected the standard errors using a quasi-GLM model, where the variance is a product of the mean and dispersion parameter. Model simplification and testing were done by deletion of terms and comparing changes in deviance using F- tests (Zuur *et al.*, 2009).

Missed or undetected species are universal problems in biodiversity studies due to imperfect detection probabilities (Pollock *et al.*, 2002; Pellet & Schmidt, 2005). In order to account for this, I used the Species Prediction and Diversity Estimation programme (Chao & Shen, 2010, SPADE) to estimate species richness. I used Abundance-Based Estimates [ACE] to compare species richness between habitat types and plot types (Chao & Lee, 1992; Chao, 2005). The total dataset was initially split into two groups, "rare" and "abundant", using the default cut-off point (10 individuals) to differentiate between them for the initial analysis. I did this because SPADE only uses the frequency statistics of rare species to estimate number of undetected species: abundant species are detected too frequently to provide information on missing species (Chao & Shen, 2003). My calculation for missing species was based on the original proposal by Turing (Chao & Shen, 2003), where the proportion of known singletons (species only found once) is the same as the fraction of unrepresented species in the sample. This estimator for missed species (C) is then added into the estimator for the total number of species.

Chao and Shen (2010) recommend the use of the default cut-off point if the ACE value is larger than the Chao1 estimate. The results from our initial analysis indicated that the ACE value was indeed higher than the Chao1 estimate for all my samples (see Results), therefore 10 was retained for subsequent analysis. Because the greater the value of the coefficient of variation (CV) for rare species the more heterogeneous the sample, models based on homogenous distributions (ACE) can significantly underestimate the species richness of a site. I classified samples with CVs greater than 0.8 as highly heterogenous and used ACE-1 as an alternative measure of species richness for those sites (Chao & Shen, 2010).

Community composition data were transformed into a distance matrix using Bray-Curtis as a distance measure to illustrate habitat-specific amphibian community assemblages and associated macrohabitat characteristics Keller *et al.* (2009). I pooled data from both years and used a non-metric multidimensional scaling analysis using PC-ORD version 5 (MjM Software, Oregon, USA). Data were separated by plot type for analyses, and outputs grouped species into assemblages representing co-occurring

species associated with given axes. 250 iterations and 250 runs of real and randomized data were generated to produce the final ordination of minimum stress, and the best fit for all three plot types. P-values were not used to interpret environmental differences among habitat types as ordination scores violate the assumptions of independence. Instead, I used correlation coefficients between each site and their corresponding axes to evaluate relationships between individual variables and ordination axes. These correlation coefficients express the rank (tau) and linear (r) relationships between the variables and ordination scores. Strong correlations (positive or negative) are represented as vectors on the ordination plots. Variables and species with r^2 values or tau numbers greater than 0.4 or less than -0.4 were considered to be, respectively, positively or negatively correlated.

I used a variant of the traditional multivariate analysis of variance (MANOVA), known as permutation-based multivariate analysis of variance (PerMANOVA) developed by Anderson (2001) to test the hypothesis of no difference between community composition between forest and plantation sites. In contrast to traditional MANOVA, PerMANOVA has the advantage of not requiring distributional assumptions such as normality and homogeneity of variance, which is rarely met in ecological community data. The same distance matrix used for the ordination was used for analysis, comparing community composition between overall habitat types and between sites within the same habitat type. PerMANOVA was done using the adonis function in R (R Developmental Team, 2012, `vegan`). This method partitions sums of squares of multivariate data sets and allows for the use of non-euclidean distance measures. The pseudo F ratios from PerMANOVA calculations were obtained without calculating the central location of groups (centroid). This is important as the centroid does not necessarily represent the central tendency in a non-euclidean space (McCune *et al.*, 2002). A total of 1000 permutations of raw data were used to obtain p- values.

2.3 Results

Mean nightly temperature did not differ significantly among habitat types in either year (Pairwise Wilcoxon test 2009: W=121, p =0.383; 2010: W= 162, p = 0.098) except for forest sites which were moderately warmer in 2010 (Pairwise Wilcoxon test: W=67.5, p = 0.026). Mean nightly rainfall also did not differ significantly among habitat types in either year (Pairwise Wilcoxon test 2009: W=1176, p = 0.6; 2010: W= 1486, p = 0.835).

Amphibian abundance did not differ significantly between habitat types (GLM; $F_{(45,44)} = 0.752$, p = 0.391), with mean abundance between two habitat types similar overall (forest =10.4 [95% CI 3.16, n=20]; plantation = 18.8 [95% CI 8.19, n=26]. When plot type (stream, riparian and terrestrial) was added to our explanatory variables, I found a significant interaction between plot type and habitat type (GLM; $F_{(40,42)} = 3.45$, p = 0.04). Mean abundances of amphibians in plantation streams were lower compared to forest streams, while mean abundance was greater in plantation terrestrial plots compared to their counterpart in secondary forest (Fig. 2.3a).

Species richness did not vary significantly among habitat type (ACE, forest: 22.2 [95% CI 0.613, n=20]; plantation: 22.4 [95% CI 0.829, n=26]) and the coefficient variation (CV) was not large enough to deem the two habitats as heterogeneous. When the data was broken down into plot types, the 95% confidence intervals of estimates of species richness overlapped among different plot types across habitat types, indicating a lack of clear difference (Fig. 2.3b).

NMDS axes cumulatively accounted for 75% of the overall variation in community composition in stream plots (minimum stress = 11.5), 78% in riparian plots (minimum stress = 13.9) and 65% in terrestrial plots (minimum stress = 17.6). Both axes for the stream plot ordination contributed equivalently to variation, while NMDS Axis 2 accounted for the majority of the variation in both riparian and terrestrial plots (Fig. 2.4-2.6). Axis 1 of the NMDS ordination for stream plotsrepresented a gradient of

decreasing canopy closure and increasing values of water quality measurements (pH, total dissolved solids, conductivity and temperature). Streams in plantation sites had higher conductivity, water temperature, greater amounts of total dissolved solids and higher pH than those in forested sites. There were no strong associations with any of the measured habitat variables among forest plots. However, the two different forest sites were separated along the 2^{nd} axis, representing the percentage of cobble (r-squared= 0.505, tau = -0.483) and dissolved oxygen content in the water (r-squared= 0.501, tau = -0.473) (Fig. 2.4).

Although the species richness was similar between forests and plantations, the community composition was strikingly different between the two habitat types (PerMANOVA, $Pseudo - F_{(1,14)} = 5.94$, p <0.01). For example, *Hylarana erythrea* and *Fejervarya nicobariensis* were both strongly associated with plantation streams, while *Phrynoidis aspera* and *Odorana hosii* were predominantly found at forest streams. There was a weaker, but still significant difference between forest streams ($Pseudo - F_{(1,6)} = 11.5$, p = 0.028): *Hylarana labialis* were more abundant in Kuala Lompat streams than in Bukit Rengit streams (Fig. 2.4).

NMDS 2 represented a gradient of increasing number of canopy closure and woody debris (small logs, percentage of leaf litter and leaf litter weight) in riparian plots. Forest plots had increased values for all these variables, while plantation plots exhibited decreased amounts of woody debris and decreased canopy closure and these differences were significant (PerMANOVA: $Pseudo - F_{(1,10)} = 6.11$, p = 0.002): *Fejervarya cancrivora* and *Fejervarya limnocharis* were the most abundant species in plantations, while abundant forest species included *Hylarana labialis* and *Hylarana glandulosa* (Fig. 2.5). In contrast to stream plots, riparian plots were not different amongst forest sites ($Pseudo - F_{(1,4)} = 4.51$, p = 0.092), however, riparian plots in plantation sites were ($Pseudo - F_{(2,6)} = 2.32$, p = 0.022). Specifically, Sungai Mai sites were separated from the other two plantation sites ($Pseudo - F_{(1,7)} = 2.91$, p = 0.015) and the abundance of *Kaloula pulchra* and *Duttaphrynus melanostictus* were greater at Sungai Mai.

Terrestrial plots in forest also exhibited increased canopy cover and leaf

litter with respect to oil palm, but also increased density of both small and large trees. Abundant species found in plantation terrestrial plots were *Fejervarya limnocharis*, a species also found in abundance in riparian plots and the rhacophorid *Polypedates leucomystax* (Fig. 2.6). Again, these differences were significant between habitats (PerMANOVA, $Pseudo - F_{(1,10)} = 3.62$, p = 0.001), but not within habitats (forest: $Pseudo - F_{(1,4)} = 2.15$, p = 0.099, plantation: $Pseudo - F_{(1,4)} = 1.11$, p = 0.494). I only found one species (*Microhyla butlerii*) present in both plantation and forest and only in terrestrial plots.

2.4 Discussion

As human-altered habitats become dominant, informative studies to aid conservation schemes, including habitat management, are urgently required. The importance of oil palm as a source of revenue in countries like Malaysia means that plantations will be a persistent part of that landscape and must be included in local conservation planning. The intense agricultural practices associated with oil palm are linked to radical loss of biodiversity in a wide spectrum of animal taxa (Chung *et al.*, 2000; Glor *et al.*, 2001; Koh & Wilcove, 2008; Brühl & Eltz, 2010). It is possible that some species within a taxa may not respond so directly when forests are converted to oil palm and for these species, managed oil palm could sustain them to some degree. The inconsistent responses of certain amphibian species from North American and Australian to serious habitat alteration suggest that these species may be good candidates for investigating the potential of oil palm as a habitat.

To do so, the use of appropriate methodology is vitally important. Many authors have commented on the problems of decreased detection probability and false negatives influencing monitoring studies (Dodd JR & Dorazio, 2004; MacKenzie & Royle, 2005; Royle, 2006). This is certainly the case for amphibians, which are small and cryptic (Sewell *et al.*, 2010; Schmidt, 2003). Even with extensively repeated sampling, individuals and even species can be missed, which may go some way to explaining the reduced abundance of frogs detected at non-stream sites in secondary

forests. In contrast to other surveys based in Malaysia, I failed to detect any Rhacophoridae species in our forest sites, but did detect microhylids (Gillespie *et al.*, 2012; Onn *et al.*, 2010). This does not imply that rhacophorids are not present in our area, as species such as *Nyctilus pictus* and *Rhacophorus appendiculatis* are known to occur. Nor does it mean that microhylids are absent in others, as tropical leaf litter frogs are notoriously difficult to detect, even with extensive surveys (Veith *et al.*, 2004) and forest floors are far more complex than oil palm plantation floors. Nevertheless, labour-intensive surveys can be impractical for wildlife monitoring, abundance only provides one metric of biodiversity and intensive surveys may actual distort estimates of abundance (Kéry *et al.*, 2009). The use of techniques that provide an estimate of the proportion of missed species, such as SPADE, may be better suited to cases where rapid, but still meaningful comparisons of biodiversity metrics like species richness are appropriate. I suggest that future studies investigating how habitat alteration affects Malaysian amphibians should avoid count statistics that fail to take missed species into account and should design future monitoring schemes to measure amphibian community composition.

Scenarios outlined in the introduction involve differences in amphibian abundance, species richness, community composition and species/habitat associations that cannot be compared using single estimators. Furthermore, surveys designed to measure differences at a gross habitat scale may not be suitable for detecting differences at the sub-habitat level, nor be effective at linking differences to specific habitat features. All of these points are illustrated by our study. I found comparable species richness and abundance in oil palm and secondary forest, yet abundance varied significantly at finer habitat scales: for example, abundance was impoverished in plantation streams when compared to forest streams. Moreover, the congruence in abundance and richness between habitats did not reflect the stark difference in amphibian community structure. Oil palm plantations were dominated by species documented to be disturbance-tolerant (Gillespie *et al.*, 2012; Wu *et al.*, 2006; Sheridan, 2003) and the oil palm community exhibited little, if any, overlap with the communities described

for secondary forests, with only one species (*Microhyla butlerii*) found inhabiting both habitats. This differs from the recent study by Gillespie and colleagues (2012), who failed to detect any microhylid species in oil palm plantation sites. This disparity between studies may be due to study location, as the common microhylid species found in oil palm plantations in Peninsular Malaysia (*Microhyla heymonsi* and *Microhyla butlerii*) are not included in Bornean checklists (Sheridan *et al.*, 2012; Inger & Lian, 1996). The relative abundance of microhylid species in Peninsular Malaysian oil palm contributed to the comparable species richness and abundance estimates among habitat types. By contrast, Gillespie and colleagues (Gillespie *et al.*, 2012) found that species richness estimates were lower in oil palm plantations than in secondary forest in Borneo. This difference between the two studies suggests that the impact of oil palm conversion on amphibian communities may not be generalized across Southeast Asian habitats.

My results support the third scenario outlined in the introduction, where habitat conversion eliminates the existing amphibian community but enables the colonization of disturbed habitat by species that can prosper in the newly created niche space. The ubiquity of these species across Malaysia (http://amphibia.my/index.php) and other parts of South East Asia indicates that the substantial amphibian biodiversity in oil palm represents a community of little conservation value, if the presence of endemic, forest species is the yardstick of "conservation value". Notably, though, amphibian community structure in plantations still had the capacity to vary in the riparian zones, while not in the forest. If community unpredictability is considered one measure of biodiversity, the riparian of oil palm is variable in a manner similar to streams located in secondary forests that, overall, appear to have greater conservation value.

In oil palm, variation in some habitat characteristics appears homogenized (e.g., forest structure, simplified stream substrate, and decreased availability of leaf litter and woody debris) or altered in a manner that should directly influence air and water temperature (opened canopy). The plantations I surveyed were converted to oil palm before the establishment

of riparian zone legislation, which now requires plantation owners to maintain a riparian vegetation strip along streams. The lack of deep-rooted riparian vegetation means that plantation streams are more straight-sided and due to high rates of sedimentation, are prone to flooding events. Plantation owners rely on mechanical dredging to relieve water pressure during the rainy season (A. Faruk, *pers. obs.*), which removes larger stream substrate and vegetation matter that is deposited in the stream. This extreme disturbance regime may explain reduced frog abundance at plantation streams and why the species that are detected are disturbance specialists. There are a number of land-management practices put in place to cope with the effects land changes have on local biodiversity, including undisturbed wildlife strips along farms, riparian buffers, pond constructions and tunnels under roads for migrating amphibians (Woltz *et al.*, 2008; Perry *et al.*, 2011; Garcia-Gonzalez & Garcia-Vazquez, 2010). Based on this study, plantation streams should be a target for future management, through the maintenance of stream complexity and riparian buffers. At the very least, given the reduction in frog abundance in plantations streams, it would seem prudent to ascertain in the future if plantations with riparian buffer zones harbour greater numbers of frogs, or support more forest-associated species.

It is uncertain how the riparian and terrestrial areas of oil palm may be managed for meaningful amphibian biodiversity. Oil palm harvesting practices require frequent disturbance and plantations are planted as monocultures and I can see little chance of manipulating these practices in a way that would encourage forest-specialist amphibians to utilize oil palm. Ultimately, even if plantation stream management proves to be successful, management of oil palm must still be accompanied with the maintenance of forest reserves to ensure amphibian conservation in Malaysia.

Figure 2.3: Barchart of mean abundance counts and estimated specie richness of amphibians in forest and plantation. Figure 3(a) is the mean frog abundance in forest and plantation habitats averaged across years for each plot type (stream, riparian and terrestrial). Figure 3(b) Estimated species richness in forest and plantation across different plot types (stream, riparian and terrestrial plots) averaged over the two years. Data pooled for all sampling plots. Estimates are based on calculations made using the Abundance-based Estimates (ACE). Solid lines in each figure represent one standard error.

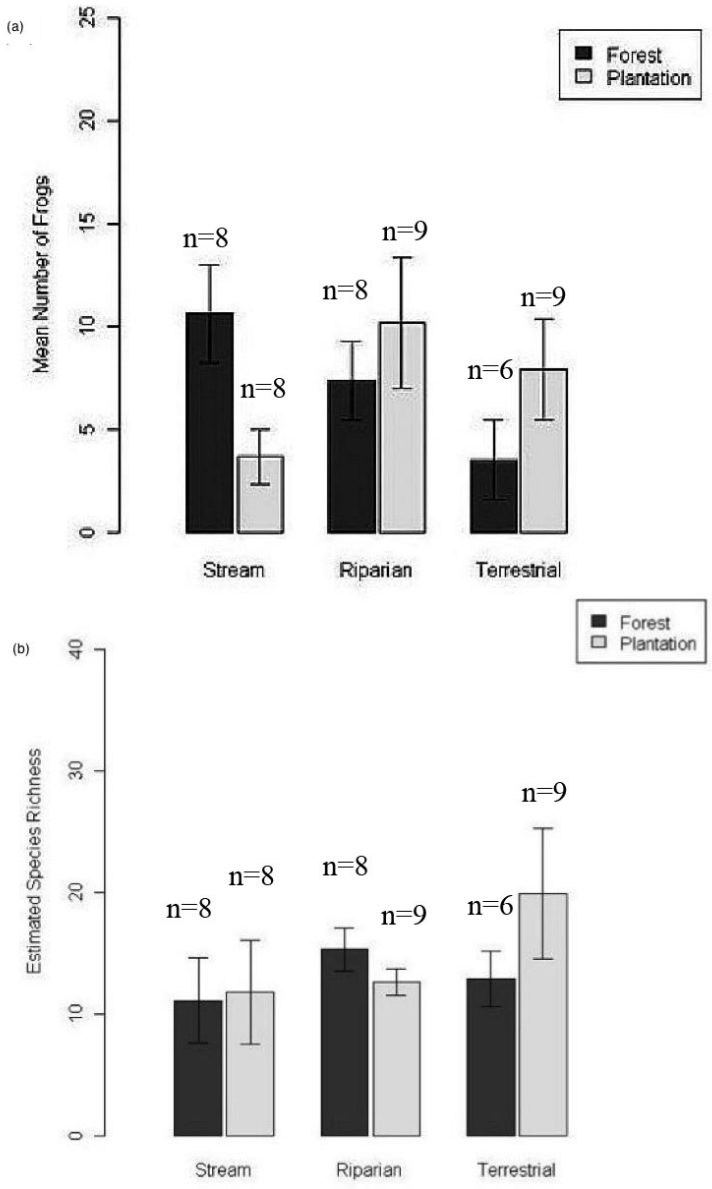


Figure 2.4: Non-metric Multidimensional Scaling (NMDS) of amphibian assemblages in stream plots in forest (BR and KL) and plantations (SM, LAN and KER) for both years. Triangles represent sites and the distance on the ordination reflects dissimilarities in amphibian species composition based on Bray-Curtis coefficients. Filled squares represent species found only in forest sites, filled circles represent species only found in plantations and filled star represent species found in both forest and plantation; refer to Table A.1 for full species names and its corresponding abbreviations. Vector loadings are macrohabitat variables with strong associations (see text): canopy = % canopy cover, Conductivity (S/m), Temperature (degrees Celcius), Cobble= % cobble on river bed, oxygen = dissolved oxygen content (ppm), TDS= Total dissolved solids (ppm), pH = water pH.

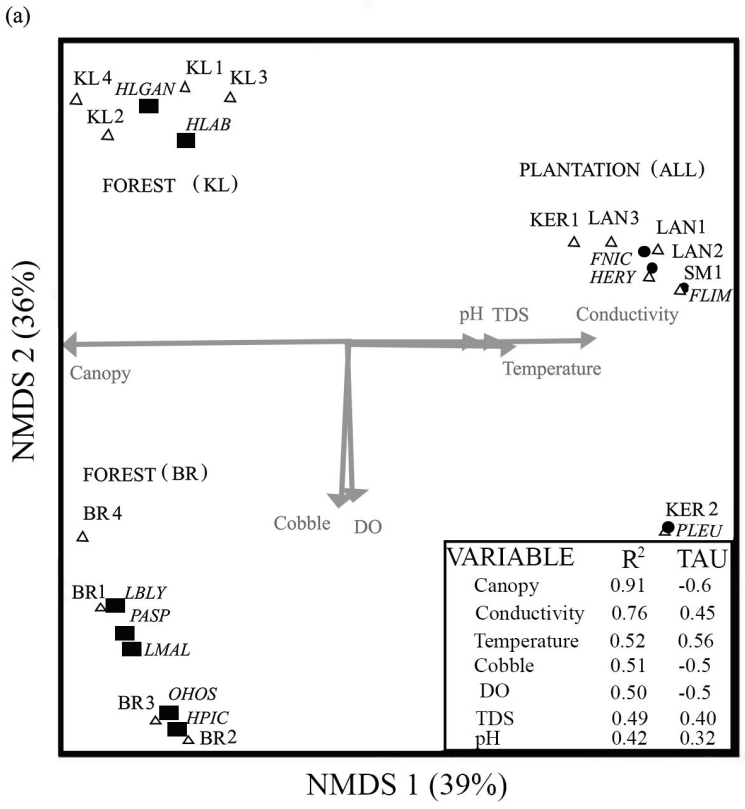


Figure 2.5: Non-metric Multidimensional Scaling (NMDS) of amphibian assemblages in riparian plots in forest (BR and KL) and plantations (SM, LAN and KER) for both years. Triangles represent sites and the distance on the ordination reflects dissimilarities in amphibian species composition based on Bray-Curtis coefficients. Filled squares represent species found only in forest sites, filled circles represent species only found in plantations and filled star represent species found in both forest and plantation; refer to Table A.1 for full species names and its corresponding abbreviations. Vector loadings are macrohabitat variables with strong associations (see text): log<50m = amount of small logs, litter = % leaf litter, canopy = % canopy cover, leaf wgt = weight difference between wet and dry leaf litter (g).

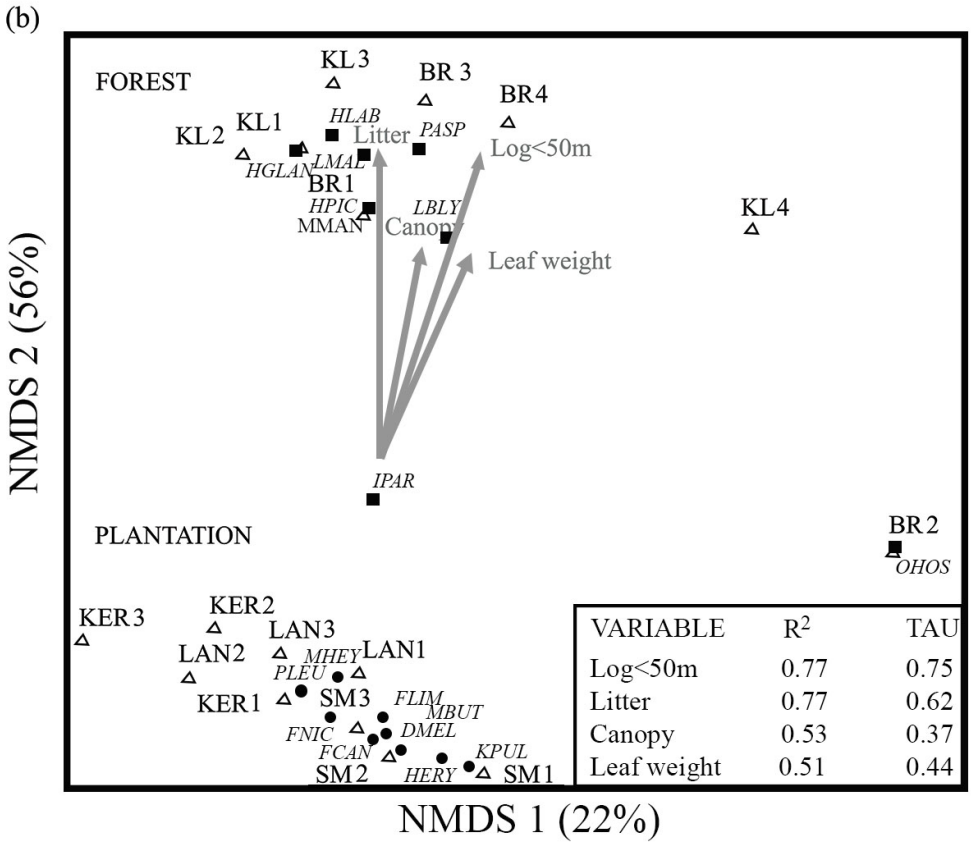
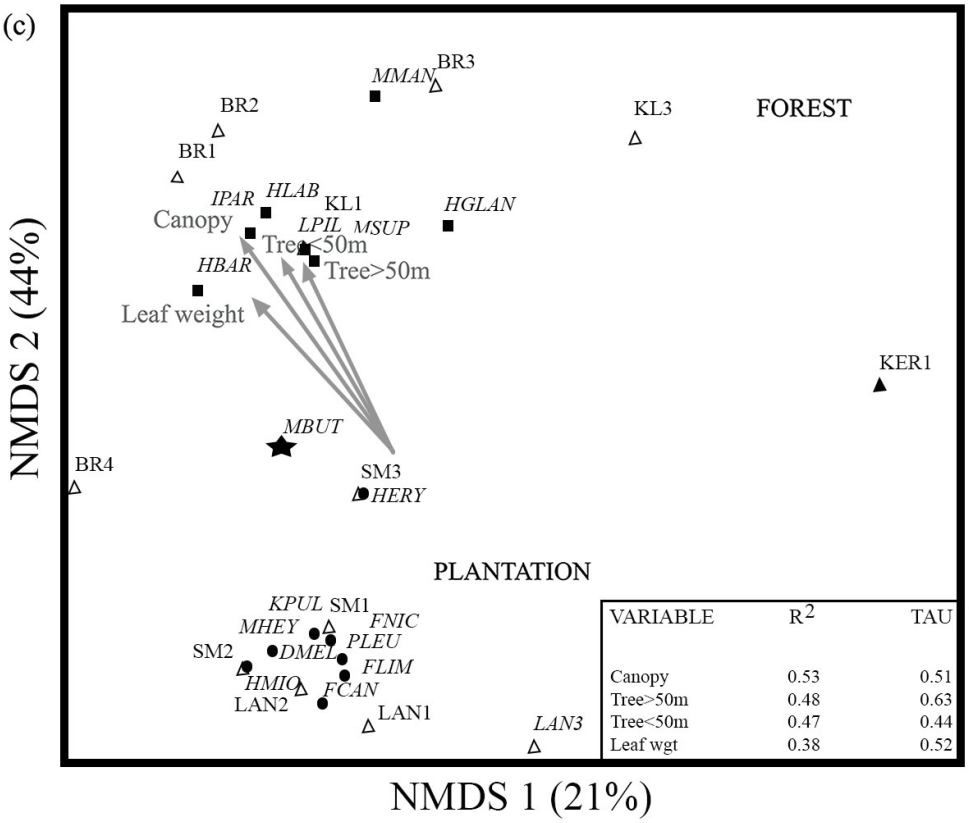


Figure 2.6: Non-metric Multidimensional Scaling (NMDS) of amphibian assemblages in terrestrial plots in forest (BR and KL) and plantations (SM, LAN and KER) for both years. Triangles represent sites and the distance on the ordination reflects dissimilarities in amphibian species composition based on Bray-Curtis coefficients. Filled squares represent species found only in forest sites, filled circles represent species only found in plantations and filled star represent species found in both forest and plantation; refer to Table A.1 for full species names and its corresponding abbreviations. Vector loadings are macrohabitat variables with strong associations (see text): canopy = % canopy cover, tree>50m = number of large trees, tree<50m = number of small trees, leaf wgt = weight difference between wet and dry leaf litter (g)) with length and angle reflecting the strength and direction of association.



Chapter 3

Suitability of temporary pools in oil palm plantations for breeding amphibians

3.1 Introduction

The rapid rise in the human population has led to a rise in food demands, which inevitably results in a growing conflict between agriculture and biodiversity. This is because conversion of natural forested habitats to agriculture has been shown to decrease biodiversity (Koh & Wilcove, 2008). It was estimated that 55% of agricultural land created between 1980 and 2000 came at the expense of intact tropical forests (Gibbs *et al.*, 2010) and in much of Southeast Asia, agricultural landscapes are rapidly becoming a dominant feature (Sheridan, 2009). In addition to species loss and decreased abundance, changes in the overall landscape have also resulted in shifts in local community composition. Survey studies looking into these altered habitats consistently find that the dominant faunal community is similar to those in other disturbed habitats (Levin *et al.*, 2006; Faruk *et al.*, in press).

The observed relationships between habitat alteration and community structure should, at least in part, relate to individual species habitat requirements and their degree of habitat specialization. Some authors have described what could be a general relationship between habitat specialization and habitat disturbance, with more specialized species tending to inhabit less disturbed landscapes (Devictor *et al.*, 2009). As specialist

species require specific habitats for successful growth and reproduction, they should be found more consistently, in greater numbers, and breeding more reliably in the optimal habitat (Duellman & Trueb, 1994; Wells, 2007). In contrast, generalist species are detected across a range of habitats and should, by definition, exhibit relatively consistent fitness across this range. The term specialist and generalist, as outlined here, are, however, trait dependent. Certain species can exhibit specialized foraging behaviour but adopt generalist strategies when selecting breeding sites or mates (Bernays, 1998), or vice versa (Thompson & Pellmyr, 1991). Accordingly, species that may be able to tolerate extreme environmental conditions associated with a converted habitat may not strictly be generalist. Strong associations with components of the altered habitat would result in these species exhibiting increased fitness despite some degree of specialization and habitat alteration. Distinct communities between agricultural and undisturbed habitats (Gardner *et al.*, 2007b; Brühl & Eltz, 2010), may also indicate that species in disturbed areas are specialized within the altered ecosystem.

Determining if a particular species is a specialist is far from straightforward. This is exacerbated when trying to identify specialism in amphibians, because they exhibit different degrees of ecological tolerance between species (Toft, 1981; Schiesari *et al.*, 2003; Wu & Kam, 2009). Species with complex life histories, however, have two distinct stages and either one or both can exhibit specialization. For example, adults of a species can show specialization to a particular habitat, but the tadpoles may be able to utilize a range of water bodies. As a result, the tadpoles location is a consequence of the adults specialization. However, tadpoles can show specialization within its current environment; in the water body. Thus, being specialized is trait-dependent and may be specific to the life stage of each species. Therefore, as well as having one being a specialist over the other, it is also possible to have either both adult and tadpole, or neither adult nor tadpole, being specialized in their respective environments.

In the Old World tropics, the majority of amphibian species rely on the aquatic environment for breeding. Temporary pools serve as an ideal breeding habitat for native amphibians because their ephemeral nature

makes them unlikely to support large predators, such as fish (DiMauro & Hunter, 2002). Variation exists between pools in terms of their size, the duration of water retention (hydroperiod), water quality and food availability. This variation can be extreme, as a consequence of location (e.g. water chemistry of a pool in fertilized farmland versus one located in low nutrient grasslands), or subtle, as a result of initial formation (e.g. pool sizes within the same wetland). The environmental structure of the pool can dictate the types of amphibians using the pools. For example, pools with very short water retention would typically be used by species that either naturally have short developmental times, or are capable of developmental plasticity in response to desiccation (Wellborn *et al.*, 1996; Skelly *et al.*, 2002). Food type and availability are also important factors in determining the suitability of pools to certain types of tadpoles (Indermaur *et al.*, 2010). As a result, certain amphibian species may depend on specific pools with environmental factors that relate to the traits of that species. Therefore, any habitat alterations that affect temporary pools can alter the types of amphibians present within the altered habitat (Meegaskumbura *et al.*, 2002).

In the previous chapter I described the high abundance of terrestrial amphibians in oil palm plantations, along with substantial differences in community composition between habitat and plot types. In this chapter, I aim to identify if the species dominating oil palm plantations are generalist within their chosen habitat, or if they show a degree of specialization in the context of their breeding site choice. If amphibians inhabiting oil palm plantations exhibit non-selective breeding site behaviour, there is the risk that certain pools can act as ecological traps (Denver *et al.*, 1998; Blaustein *et al.*, 1999; Bancroft *et al.*, 2008). The close proximity of plantations to forested areas provides the opportunity to identify the effects of intensive agriculture on the frequency and structure of temporary pools. I began by identifying the differences between temporary pools located in the plantation and those in forest habitats. This was followed by a comparison of the proportion of pools used by amphibians in the two habitat types. I also compared the types of species found breeding in temporary pools between habitat types, identified tadpole feeding guilds and determine

if temporary pools in plantations are able to support tadpoles typically found in forested habitats. Finally, I tested the disturbance-generalist paradigm, by identifying if temporary pool breeding amphibians in plantations show specificity in breeding site preferences, relating to variables that are specific to oil palm plantations. I hypothesize that the exposed nature of oil palm plantations would allow for an increase in temporary pool formation. Although an increase in pool frequency would, inevitably, increase the number of potential breeding sites for plantation amphibians, the open canopy in plantations could also result in a wide range of variation between pool structure and water chemistry. A strong variation between pools may increase the probability of individuals encountering pools that are unsuitable as breeding sites and therefore, these pools can potentially act as ecological traps within the habitat. In terms of usage, I predict that plantation pools will have high occupancy, possibly similar to forest pools, and that temporary pool breeding amphibians in plantations would show preferences to specific pools for breeding.

3.2 Method

Site description

This study was conducted in the district of Temerloh within the state of Pahang, Peninsular Malaysia (3°40'N, 102°10'E). Two mature (>30years) oil palm plantations (Lanchang and Sungai Mai) and two secondary forest sites (Bukit Rengit and Kuala Lompat) within the Krau Wildlife Reserve were chosen for this comparative study. A full description of the study area is outlined in Chapter 2 of this thesis.

Field surveys

Initial searches for temporary pools within the two habitat types were made at the beginning of May 2010. Repeated visits were conducted every week after that for five weeks. Searches were made during the day along a 2km path within each study site (Lanchang, Sungai Mai, Bukit

Rengit and Kuala Lompat). Each 2km path started 500m from the entrance of each study site; 500m from the entrance into each plantation site and 500m from the beginning of the trail into each forest site. Each pool, located up to 5m on either side of the 2km path, was given a unique number and its location recorded using a hand-held GPS. The physical structure (depth, length and width, estimated percentage leaf litter cover and average canopy cover) of each pool was recorded during the initial search. Pool dimensions were measured using a tape measure to the nearest centimeter. During repeat visits, depth measurements were taken, as close as possible to the center of each pool, and at the same time of day, in order to quantify changes in depth.

The amount of leaf litter and/or woody debris (leaf litter cover) in each pool was estimate by visually determining the percentage of the pool covered by leaf litter and/or woody debris. In cases where pool water was too murky for visual estimation, litter cover of the area 1m from the pool waters edge was used as a proxy. Canopy cover was estimated by taking a digital photograph of the understory canopy at four positions around the pool using a Nikon D40 camera (Nikon 10.5mm f/2.8 DX fisheye lens). To identify the first position, I randomly ordered the numbers 0 to 359 and gave each pool a randomly selected number. This number was used as the first position, based on the points of a compass, from which the other four points were referenced. The second, third and fourth positions were then set at 90°, 180° and 270°, respectively, from the first position. Digital photographs of the canopy taken at all four positions were analyzed using ImageJ (Rasband, 2011) after converting each picture to a black and white 8-bit image. I set a minimum threshold value of 100 and pixels that exceeded this value were taken to represent canopy cover (black pixels). An average percentage of black pixels across the four points were used as an estimate of canopy cover for each pool. I estimated the proximity of each pool to a road by measuring the distance between the pool and the nearest, frequently used road. I identified the difference between a walking path and a vehicle road informally by questioning local guides, indigenous tribes for forest sites and plantation workers in plantation sites. For roads used by vehicles, I identified if they were used frequently by counting

the number of motorized vehicles passing the location of the pool in a 30 minute time slot. For plantation pools, observations were made during the time when palms were collected for transport out of the plantations, for forest pools, observations were made between the hours of 4pm to 7pm, corresponding to the time of heaviest traffic.

I measured the water temperature (° C) and chemistry [pH, total dissolved solids (TDS in ppm)] for each pool during the initial visit using a handheld water testing device (Hanna HI-98129 standard combo meter). The probe was completely submerged into the water and held as close to the middle of the pool as possible without disturbing the substrate. A 10ml water sample from each pool was collected and tested in the field for dissolved oxygen content [DO] (mg/l) and Nitrate content (mg/l) using a field water testing kit (TetraTest, Blackburg, VA).

To estimate occupancy/presence of adults, searches were repeated during each visit, as lack of detection during a single visit does not necessarily mean a true absence of that particular species. Where possible, adults were caught, identified in the field and released at the point of capture. Evidence of breeding (egg masses and tadpoles) was also recorded for each pool. Egg masses were collected, hatched and reared at the University Malaya laboratory for species identification. Tadpoles from the field were caught using dip nets and identified where possible. Aside from identifying species, I also determined the feeding ecology of tadpoles. For individuals that could be easily identified to species, feeding habits were determined from the literature. If the tadpole could not be easily identified or the literature lacked documentation, tadpole feeding behaviour was determined based on the structure of its mouthparts and position within the water column. The individual tadpole was sedated using a 0.1% diluted solution of MS-222 in a petri dish and its mouthparts examined under a dissecting microscope. Descriptions of mouthparts were derived from Sokol (1975). Tadpoles with simple mouthparts (i.e. a single opercular chamber and lacked keratinous parts) were classified as filter feeders, while those with keratinous mouthparts were classified as grazers. Filter feeders were further classified based on the tadpoles position in the water column during initial capture. In order to do this, each pool was carefully approached

by one person, as certain species are extremely sensitive to tremors and will swim to the bottom when disturbed. Tadpoles found just under the surface film of the water were noted as being positioned in the top section of the water column. Tadpoles that were seen amongst leaf litter or algae at the bottom of the pool were noted as being in the bottom section of the water column. Finally, those that were off the bottom but not feeding on the surface film were noted as being within the water column. Surface film feeders were typically found in the top section and general filter feeders found in the middle of the water column.

Data analysis

Water retention of pools was compared using a standard Generalised Linear Models (GLMs). I used site and visit number (1^{st}, 2^{nd}, 3^{rd} and 4^{th}) as predictor variables and water depth measurements per visit as the response variable. Water depth data were log transformed before generating models to ensure normality. Model simplification was done by sequentially dropping each predictor variable without replacement and testing the new model using F-tests.

I estimated pool surface area assuming an oval shape and using the following equation:

$$Area = \frac{4}{5} \times (Width_{max} \times Length_{max}) \tag{3.1}$$

Pool area, along with the other physical variables, except maximum width and length, were compared between oil palm and forest using Wilcoxon rank sum test. The same statistic was used for all the chemical variables measured during the field season. A normal approximation of p-values was computed in the event of ties. The degree of variation in pool structure and water chemistry was compared between habitat types using an F-test.

Recent literature has suggested the importance of incorporating detection probabilities in estimating the presence or absence of species and/or populations of amphibians (Dodd JR & Dorazio, 2004). The following is a simple formula describing the relationship between the number of observed

species (C) and the true number of species (N):

$$C = Np \tag{3.2}$$

where p is the detection probability. According to the formula, when p is variable and/or less than 1 (imperfect), which is frequently the case for ecological surveys, the value of C needs to be adjusted in order for the value of N to be valid (Schmidt, 2003). Amphibian occupancy and detection probability of forest and plantation pools were estimated using the *occu()* function from *unmarked* package in R (R Developmental Team, 2012). This particular package allows for the use of hierarchical models, which accounts for spatial/temporal variation in the collected data. The function is based on the occupancy model developed by MacKenzie and colleagues (2002), and fits the observational model below:

$$y_i|z_i \sim Bernoulli(z_i p) \tag{3.3}$$

where the observed presence/absence (y) at site i, conditioned to the state variable or true presence/ absence of the species or individual z is a product of the true state and detection probability p under a Bernoulli model (coin toss). Whether or not a spatial unit is occupied is based on the following process model, which is also a Bernoulli model:

$$z_i \sim Bernoulli(\psi_i) \tag{3.4}$$

which can be modelled using covariates (x) in a logistic equation to estimate occurrence probability ψ:

$$logit(\psi_i) = \alpha + \beta(x_i) \tag{3.5}$$

where α and β are parameters from the fitted model.

The use of covariates is based on the idea that one would expect occupancy to be a function of some measured site characteristic and detection varying with a certain measured variable, such as daily weather characteristics. The inclusion of covariates allows for the assumption that occupancy and detection are constant across sites (MacKenzie *et al.*, 2002).

Models that assumed constant occupancy and detection, constant occupancy but variable detection, variable occupancy but constant detection, and models varying in both occupancy and detection were generated and compared to identify the model that best fit the occupancy data. Standard errors of occupancy and detection estimates were generated using a nonparametric bootstrap method. Comparisons between models were done based on AIC_c numbers. I used the *MuMIn* package in R to generate AIC_c values for each model, as AIC_c corrects for small sample size (Anderson & Burnham, 2002).

When models had constant occupancy/detection, true values from the resulting estimates were calculated using a simple inverse logit [*plogis()*] function. For models with covariates, the relationship between occupancy/detection with corresponding variables was plotted along with 95% confidence interval. The average values for corresponding variables were added into equation 3.5 and occupancy/detection values calculated as with the constant model.

Linear quadratic models were fitted to compare the rate of change of water depth per visit between pools used for breeding (pools where tadpoles and eggs were detected at least once) and those that were not. Additionally, generalized linear models [GLMs] with binomial errors were used to model amphibian breeding with respect to physical and chemical variables associated for each pool. Prior to fitting the maximal model, correlations between habitat variables were identified using Kendall's tau statistics. Highly correlated variables (tau value>0.8) were placed into a principal component analysis [PCA] and the main principal component [PC1] was used in subsequent analyses. Model simplification and testing was done by the deletion of the least significant variable from the maximal model and comparison of changes in deviance using Chi-square.

3.3 Results

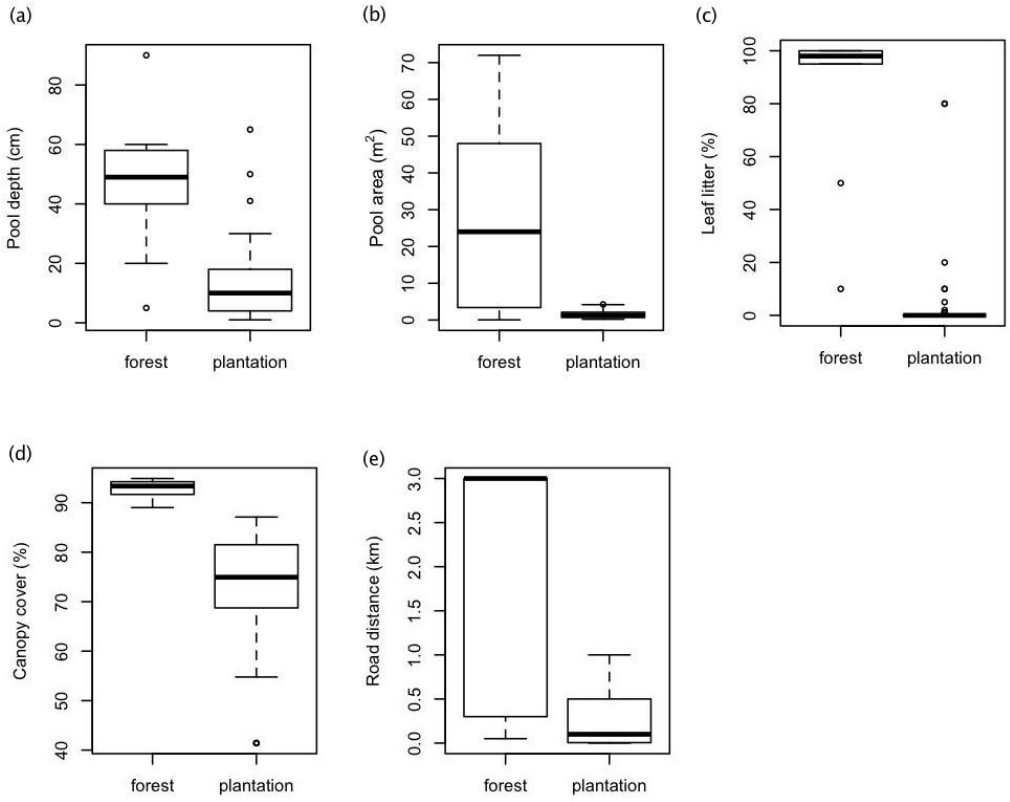
Pool characteristics

Temporary pools were detected more frequently in plantation sites compared to forest sites. A total of 45 pools were found in plantations (2.25 pools per km^2), while 9 pools were detected in forest sites (0.45 pools per km^2). Forest and plantation pools were significantly different in estimated surface area (W = 343.5, *p*-value < 0.01), percentage of canopy cover (W = 3.00, *p*-value <0.01), leaf litter cover (W = 6, p-value <0.01) and distance from roads used by vehicles (W = 339.5, *p*-value < 0.01). Forest pools were generally larger (median area: forest= 24m^2, plantation=1.28m^2), with more canopy (median canopy: forest=93.4%, plantation=74.9%), and leaf litter cover (median cover: forest=98%, plantation=0%) and were located further from active roads. The only structural variable that did not vary significantly between the two habitat types was pool depth (W = 174, *p*-value = 0.513; Figure 3.1).

Forest pools had greater variation than plantation pools in area ($F_{(8,44)}$ = 623, *p*-value <0.001) and distance to active roads ($F_{(8,44)}$ = 11.63, *p*-value <0.001), while both canopy cover ($F_{(8,44)}$ = 0.029, *p*-value <0.001) and leaf litter cover ($F_{(8,44)}$ = 3.546, *p*-value = 0.006) were more variable for plantation pools (Figure 3.1). Site was the best predictor of water retention (GLM; $F_{(14,11)}$ = 11.26, *p*-value <0.01). Forest pools retained water longer (coefficient estimates: BR=2.82; KL=0.82) than plantations pools (coefficient estimates: LAN=-0.55; SM=-0.72; Figure 3.2). As a result, more plantation pools had dried out by the end of the study (67%) than did forest pools (11%).

Comparisons of water chemistry between the two habitat types indicated no significant difference in nitrate concentrations (W=156, *p*-value =0.409) and pH (W=86, *p*-value =0.057). Dissolved oxygen content (W=104, *p*-value =0.042), total dissolved solids [TDS] (W= 57, *p*-value = 0.012) and temperature (W= 0, *p*-value <0.01) were all significantly greater in the plantation pools (Figure 3.3). TDS ($F_{(6,40)}$=0.006, *p*-value <0.01) and water temperature ($F_{(7,23)}$=0.055, *p*-value =0.001) were more variable in plantation pools compared to forest pools, while pH was more

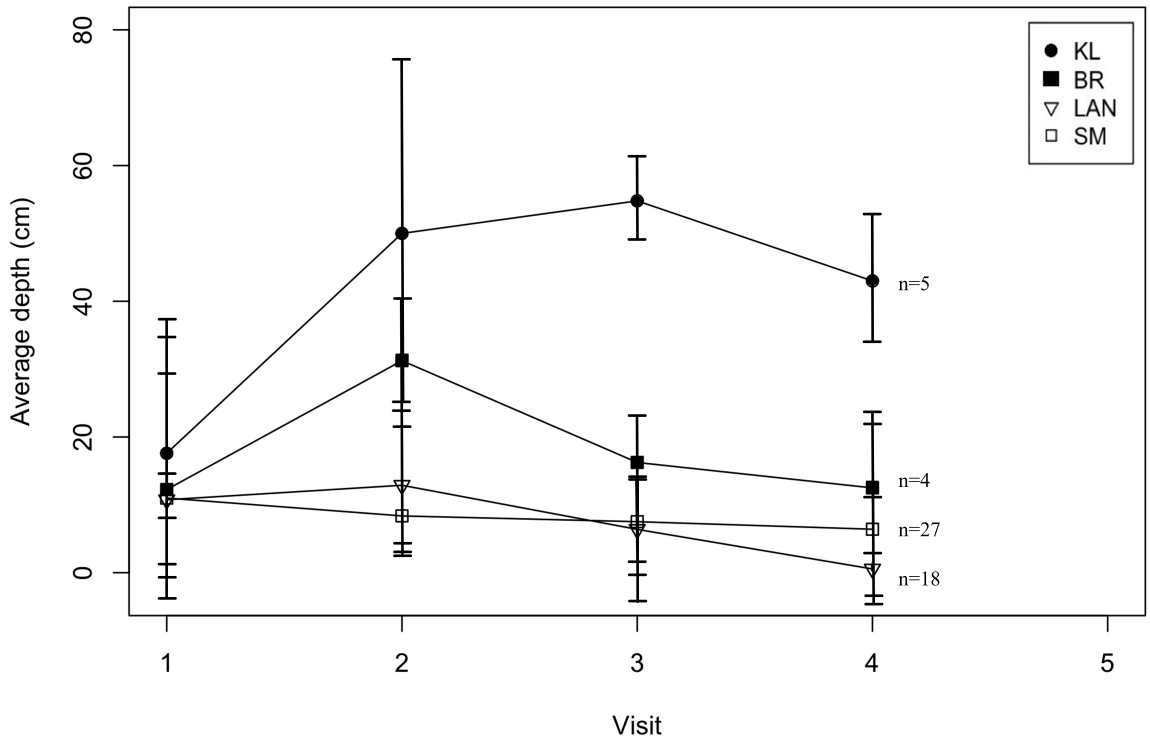
Figure 3.1: Comparison of measured physical variables between pools found in oil palm plantations and forest sites. Measurements include maximum pool depth (a), estimated pool area (b) calculated using maximum length and width measurements, percentage leaf litter cover within pools (c), percentage canopy cover over pool (d) and distance pool from the nearest vehicle used road (e). The thick line represents the median value, while the top and bottom of the boxes represent the upper and lower quantiles, respectively. The whiskers represent the spread of the data (minimum and maximum values), with the open circles representing outliers in the data.



variable in forest pools compared to plantation pools ($F_{(7,36)}$=4.279, *p*-value =0.003) (Figure 3.3).

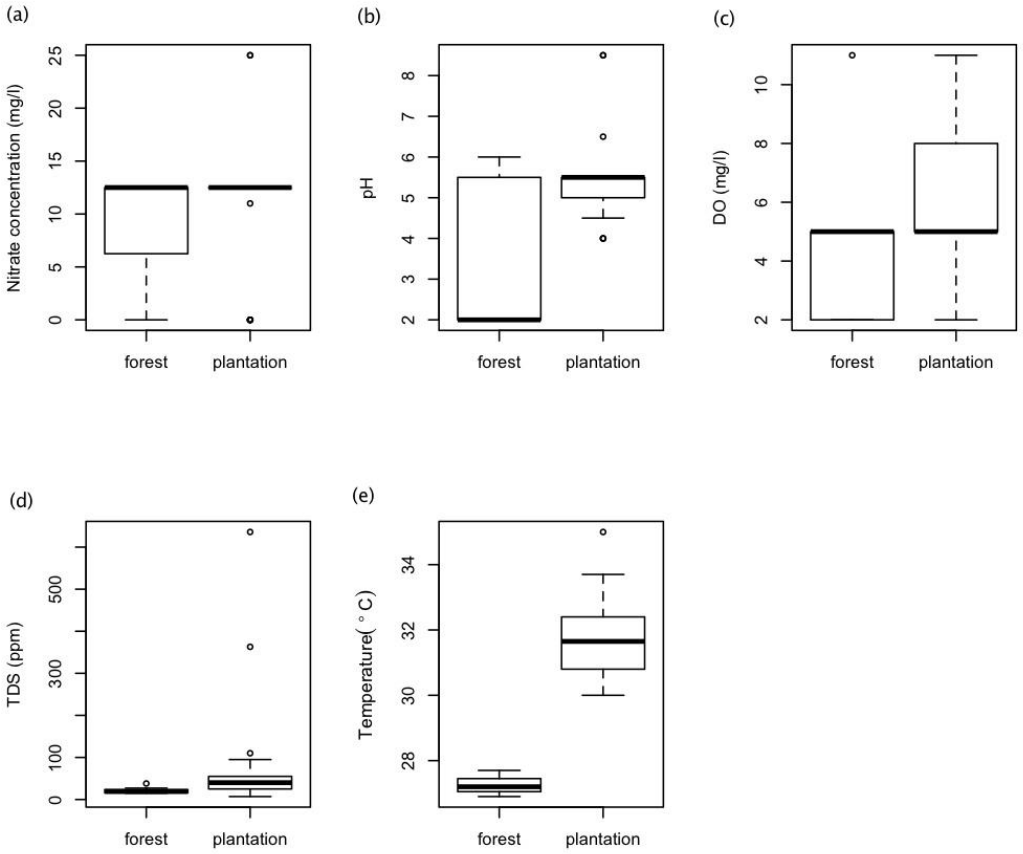
I detected a total of 12 species of amphibians from three families, either as eggs, tadpoles or adults. Two species were found in both habitat types, while other species were only found in forest sites or plantation sites. In

Figure 3.2: Average depth of temporary pools in four study sites against the number of visits. Forest sites represented by triangles (open = Kuala Lompat (KL), closed = Bukit Rengit (BR)). Plantation sites represented by circles (open = Lanchang (LAN), closed = Sungai Mai (SM))



general, all the microhylid tadpoles found during the study exhibited the simple mouthparts associated with filter feeding. Within this classification, *Microhyla heymonsi* tadpoles were separated from other species based on its position in the water column and classified as a 'surface film feeder' (Table 3.1). The mouthparts of tadpoles from the Dicroglossid family were more adapted for scraping and grazing on algae. One exception to this was *Occidozyga laevis*, which is predatory, based on gut dissections by Heyer (1973) . Another exception was *Limnonectus laticeps*, which uses a yolk sac for nutrition throughout its tadpole stage.

Figure 3.3: Comparison of measured chemical variables between pools found in oil palm plantations and forest sites. Measurements include nitrate (a), pH (b) dissolved oxygen content [DO] (c), total dissolved solids [TDS] (d) and temperature (e). The thick line represents the median value, while the top and bottom of the boxes represent the upper and lower quantiles, respectively. The whiskers represent the spread of the data (minimum and maximum values), with the open circles representing outliers in the data.



Models generated for forest pools that included water level and maximum depth measurements as covariates did not converge. As models with unconverged parameters cannot be confidently compared using AIC_c values, they were omitted. Four models with constant detection probability, one with constant occupancy and the rest with variable occupancy modeled with covariates, were compared. The model with constant detection and occupancy, $p(.)\psi(.)$ had substantially more weight than the models

Table 3.1: Species found in pools during the survey period at difference stages (Egg, Tadpole and Adult) along with their position in water column (top, within and bottom) when found and known feeding habits (SF= suspension feeder, AF=algae feeder, Pr= predatory, Y=yolk sac). Information on feeding habits are either taken from Heyer (1974) (∗) or derived from mouthpart morphology Inthara *et al.* (2005)

Species	Forest	Oil Palm	Stage	Feeding	Position
Microhylidae					
*Microhyla heymonsi**	+	+	E, T and A	SF	Top
M. superciliaris	+	-	T and A	SF	Middle
*M. butleri**	+	+	T and A	SF	Middle
M. mantheyi	+	-	T and A	SF	Middle
Kaloula pulchra	-	+	A	SF	Middle
Dicroglossidae					
Fejervarya limnocharis	-	+	E, T and A	AF	Bottom
Hylarana glandulosa	+	-	A	AF	Bottom
Occidozyga laevis	+	-	A	Pr	Middle
Limnonectus plicatellus	+	-	T and A	AF	Bottom
L. laticep	-	+	A	Y	Top
Rhacophoridae					
*Polypedates leucomystax**	-	+	E, T and A	SF	Bottom
Rhacophorus sp.	+	-	T	N/A	Middle

where occupancy (ψ) varied with covariates (Table 3.2). Straightforward back-transformation revealed that detection probability of amphibians for all forest pools was 0.917 (SE=0.057), with an occupancy estimate of 0.667 (SE=0.157). This indicated that around 67% of pools found in forest sites were occupied by amphibians with a 92% chance of detecting amphibians in those pools.

16 models were fitted for pools in plantations and ranked based on AIC_c values and model weights (Table 3.2). There was no single model with a high enough AIC_c weight therefore a conclusive statement could not be made regarding the importance of covariates used in the model. However, models with p(water) performed better than when detection was constant [$p(.)$], suggesting that detection probability varied with pool water level. Additionally, models with variable detection probabilities provided similar estimates of the overall detection rates ($\sim$ 51%). Detection

probability rose sharply in pools with water levels between 0 and 5cm, then the rate decreases to a plateau at a high probability of detection (Figure 3.3a). Standard error for detection probability tends to increase between 2-3cm pools, but tapers to almost negligible from 10cm onwards (Figure 3.3a). Although the model with leaf litter cover as a covariate for occupancy [$\psi(litter)$] had the highest AIC_c value, removing this covariate did not significantly worsen the fit of the model (Table 3.2). Furthermore, the standard error for the litter covariate model rapidly increased after 5% litter cover (Figure 3.4b), which presents uncertainty in estimating occupancy accurately. Therefore, the constant occupancy model [$\psi(.)$] was selected and based on this model amphibians occupied 64% (SE=0.09) of pools in plantations (Table 3.2).

Oviposition site selection

The fitted linear models between breeding and non-breeding pools had negative intercepts (estimated coefficients: breeding = -3.60 [se = 6.27], non-breeding = -7.55 [se = 5.83]) and showed water depth initially increased for both pools (estimated coefficients: breeding = 10.3 [se = 5.72], non-breeding = 13.7 [se = 5.32]) between the first and second visit. After the second visit, water depth decreased at a similar rate between pools (estimated coefficients: breeding = -2.13 [se = 1.13], non-breeding = -2.73 [se = 1.05]) (Figure 3.5). Both models showed an acceptable level of fit based on adjusted r^2 values, with around 36.1% and 61.7% of the variability explained for breeding and non-breeding pool data, respectively. Initially, I found a very slight correlation between the pool variables, more specifically between nitrate concentrations, maximum depth and percentage leaf litter (tau value ~ 0.3), but this was not high enough to warrant any further analysis to reduce multicollinearity. I chose not to generate models using the forest pool selection data, as the small sample size (n=9) was inadequate for analysis, therefore, only the plantation data was used for oviposition selection models.

Pool depth and nitrate concentration seemed to play an important role in breeding site choice in plantations as removal of these variables from

Table 3.2: Amphibian occupancy and detection probability models generated from *unmarked* package of forest and plantation pools. Values for occupancy (ψ) and detection (p) are back-transformed estimates from the corresponding models. Intercept values are estimates of occupancy if it were constant, similarly with detection. Table includes estimates if occupancy varies with measured variables (pool area=area, maximum water depth=depth and leaf litter cover=litter) and if detection varies with water retention (water). The degree of freedom (df) for each model, relative difference in $AIC_c(\Delta AIC_c)$ and AIC_c model weights per habitat type are also shown.

Models, per habitat type	ψ				p		df	ΔAIC_c	Weights
	(intercept)	(area)	(depth)	(litter)	(intercept)	(water)			
Forest									
$\psi(.)p(.)$	0.667	-	-	-	0.917	-	2	0.000	0.864
$\psi(litter)p(.)$	0.208	-	-	0.506	0.917	-	3	3.680	0.142
$\psi(litter + area)p(.)$	0.315	0.495	-	0.506	0.917	-	4	10.39	0.010
$\psi(area)p(.)$	0.00	1.00	-	-	0.687	-	3	19.33	0.000
Plantation									
$\psi(litter)p(water)$	0.738	-	-	0.938	0.476	0.512	3	0.00	0.326
$\psi(.)p(water)$	0.628	-	-	-	0.469	0.512	2	1.03	0.195
$\psi(area + litter)p(water)$	0.629	0.540	-	-	0.469	0.512	3	2.37	0.100
$\psi(depth + litter)p(water)$	0.629	-	0.561	-	0.470	0.512	3	2.54	0.092
$\psi(depth)p(water)$	0.634	-	0.565	-	0.468	0.512	4	3.25	0.064
$\psi(area)p(water)$	0.724	0.541	-	0.919	0.476	0.512	4	3.26	0.064
$\psi(litter)p(.)$	0.717	-	0.523	0.905	0.475	-	5	4.11	0.042
$\psi(area + depth + litter)p(water)$	0.734	0.541	0.522	0.932	0.476	0.512	4	5.04	0.026
$\psi(.)p(.)$	0.637	-	-	-	0.390	-	4	5.39	0.022
$\psi(area + depth)p(water)$	0.728	0.544	0.500	0.916	0.397	0.512	6	5.58	0.020
$\psi(area + litter)p(.)$	0.634	0.547	0.538	-	0.392	-	4	6.29	0.014
$\psi(depth + litter)p(.)$	0.634	-	0.540	-	0.393	-	5	6.48	0.013
$\psi(depth)p(.)$	0.742	-	-	0.935	0.397	-	4	7.22	0.009
$\psi(area)p(.)$	0.730	0.550	-	0.919	0.398	-	5	7.41	0.008
$\psi(area + depth + litter)p(.)$	0.742	0.548	0.500	0.936	0.396	-	5	8.78	0.004
$\psi(area + depth)p(.)$	0.637	0.556	-	-	0.388	-	3	9.30	0.003

Figure 3.4: Expected amphibian detection probability against varying pool depth (a) and estimated amphibian occupancy probability against leaf litter cover (b), based on occupancy models that best explained the absence/presence data collected from oil palm plantations only. Solid line is the fitted values of the model with the lowest AIC_c value and the highest weight. Grey lines represent the upper and lower bounds [95% CI].

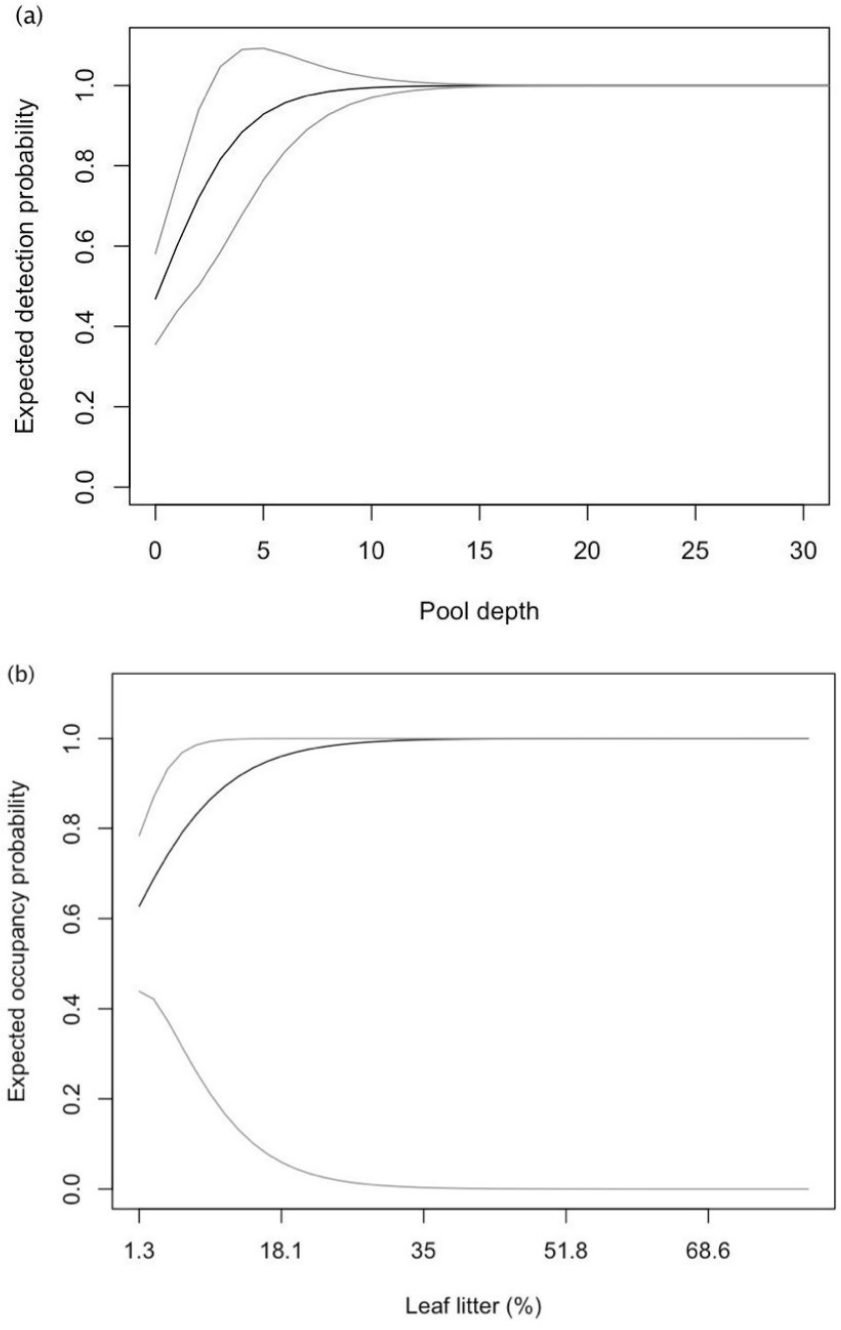
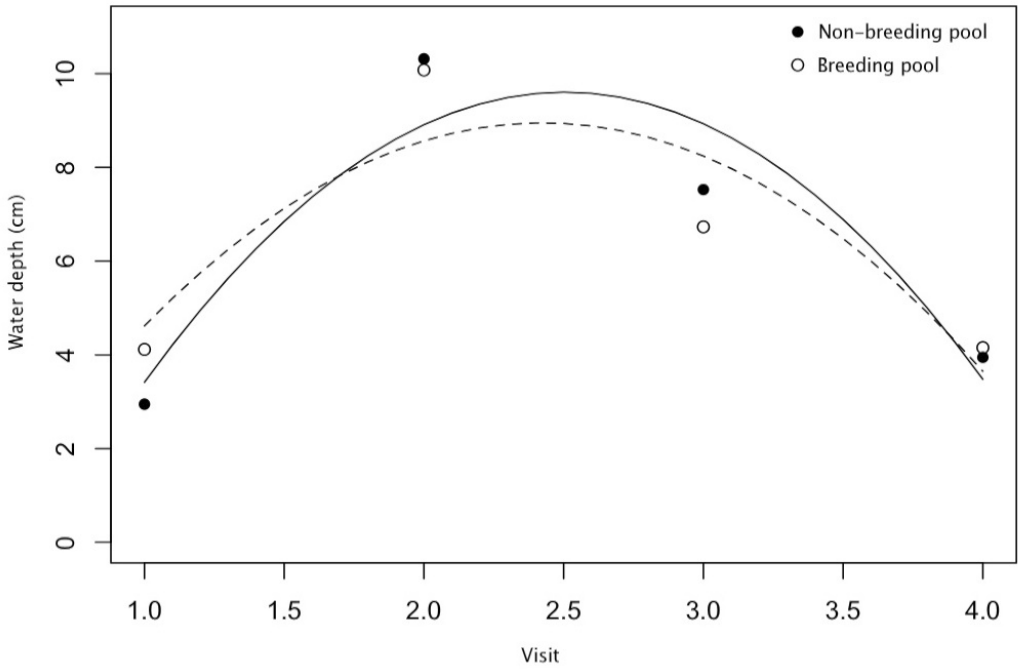


Figure 3.5: Relationship between average water depth against visits for breeding and non-breeding pools. Solid line represents the fitted linear quadratic model for change of water depth in non-breeding, while dashed lines represents the model for pools used for breeding (presence of tadpoles or eggs found during, at least, one visit). Filled and open circles are observed values.



the model led to a significantly worse fit to the data (Table 3.3). The probability of breeding at a particular pool (y) was dependent on pool depth (D) and nitrate concentrations (Ni) using the following equation:

$$y = logit^{-1}(-1.56 + 2.30 * D + 0.20 * Ni) \tag{3.6}$$

Both variables exhibited a positive relationship with amphibian breeding (Figure 3.6). The fitted curve generated against pool depth followed a typical logit distribution. At shallow depths, the probability of detecting evidence of amphibian breeding was low. Probability of detection increased rapidly up to 40cm, at which point, it plateaued with a high

Table 3.3: Model selection for evidence of amphibian breeding in pools and explanatory variables. Variables were removed from the maximal model. Model fit was tested between the new and previous models using a Chi-square test. Model deviance, Chi-square and p-values are shown in the table below. Degrees of freedom for each model = 1, 44

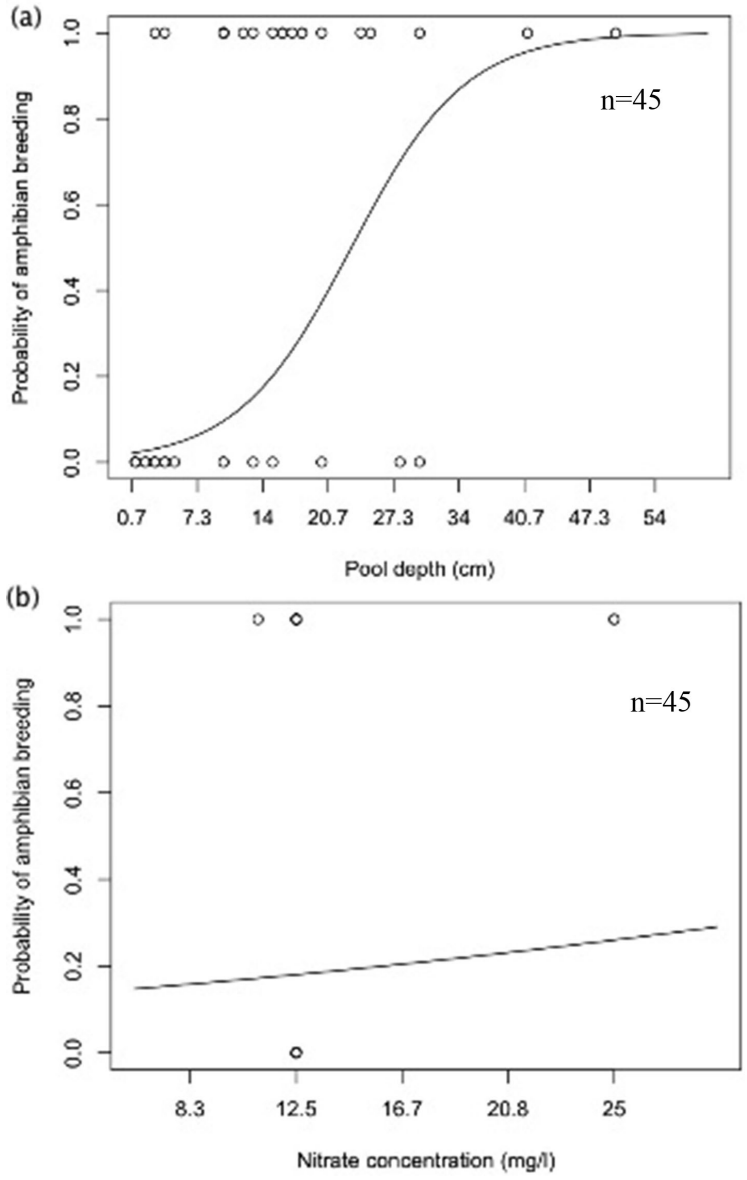
Variable removed	Model Deviance	Chi-square value	p-value
none	41.0		
area	41.4	0.00	0.549
litter	41.0	0.00	0.961
maximum depth	53.9	12.9	<0.001
road	41.0	0.02	0.890
dissolved oxygen	43.1	2.07	0.151
nitrate	45.9	4.88	0.027
ph	41.0	0.01	0.913
total dissolved solids	42.1	1.14	0.285
temperature	41.1	0.08	0.772

probability value (Figure 3.6a). The fitted curve of amphibian breeding against nitrate concentrations exhibited a shallower gradient in comparison to depth, but still showed a positive relationship with amphibian breeding (Figure 3.6b)

3.4 Discussion

The features associated with a particular body of water are largely dependent on its surrounding habitat and can vary significantly, not only between different habitats, but also between water bodies within the same habitat (Wellborn *et al.*, 1996; DiMauro & Hunter, 2002). The present study highlighted clear differences between temporary pools in forest and oil palm plantations. Pools in plantations exhibit characteristics typical of water bodies in altered habitats; small, exposed pools with poor water retention, located closer to motorized traffic, greater amounts of TDS and higher variability in water temperature and canopy cover than natural pools (DiMauro & Hunter, 2002). Characteristics of pools in plantations are consistent with the exposed nature of the habitat. For example, the

Figure 3.6: Graph showing fitted values (solid line) of the minimal model with binomial errors applied on presence of amphibian breeding data. The x-axis is pool depth measurements (a) and nitrate concentrations (b). Open circles are observed values and n is the total number of pools sampled.



reduced canopy cover in plantations, reduced leaf litter cover and higher variability in water temperature within pools. These differences of temporary pools between habitats can have an effect on correctly estimating amphibian usage, due to variation in detection. Detection probability of amphibians in plantations varied with water level, which can be a product

of the exposed nature of plantation pools. Pools in such an exposed habitat can exhibit rapid filling and drying, which can affect amphibian detection at a particular location, as breeding amphibians (either calling or ovipositing) which are located in water, are typically easier to detect than those not attending aquatic breeding sites (Pellet & Schmidt, 2005). Therefore, in order to estimate amphibian occupancy, statistical techniques taking into account varying detection probabilities are highly encouraged.

Pools in plantation sites were also more common, so amphibians in plantation sites were exposed to a greater number of physically variable pools within this habitat type. Despite these results, the proportion of occupied plantation pools was similar to the proportion of occupied forest pools. Although only a fraction of plantation pools were occupied, the physical variables I measured were not important in determining occupancy when all amphibians were considered. There are a number of possible explanations for this result, however, selectivity of pools by breeding and non-breeding amphibians cannot be conclusively argued. Usage of certain pools can be a response to an aggregated distribution of amphibians within the landscape, which is typically found in natural populations (Hayek & Buzas, 2010). Alternatively, individuals may be selecting pools based on features not measured during the study. A direct comparison of occupancy estimates between plantation and forest could not be done, as the small sample size of forest pools resulted in the non-convergence of occupancy and detection models (Anderson & Burnham, 2002). By removing these models, we may be excluding important effects of such covariates if, for example, the sample sizes were increased; we cannot be certain of constant detection in forest sites, or if increasing the sample size may reveal variation in detection, which is becoming increasingly evident in the literature (Schmidt, 2003). However, all the converged models had similar values and pools where amphibians were detected at the first visit were also detected at every consequent visit.

As mentioned in the introduction, detecting specialization by amphibians is made more difficult due to complex life histories. Although I was unable to specify the variable (or variables) associated with occupancy by all amphibians, I did find selectivity in terms of plantation pools used

specifically for breeding. Evidence of breeding was restricted to deeper pools. As the rates of water loss were similar between breeding and non-breeding pools, the importance of pool depth to the length of water retention would be more notable in a generally exposed habitat. The other significant variable explaining breeding site selection was nitrate concentrations, which is a correlate for algal growth but if amphibians actively choose pools based on this variable is unclear. Increased higher nitrate concentration could be caused by longer periods of water retention, which in turn, is associated with deeper pools. Higher nitrate concentrations could result from runoff from fertilizers and pools closer to the source would have higher concentrations. In this case increased breeding activity in pools with increased nitrate concentration is not a result of selection for nitrates and its covariates, but due to associations between amphibians and the source of fertilizer input. Despite the disparity amongst variables between forest and plantation pools, and greater variability of some of these in plantations, no other variable affected breeding site choice. The preference for deeper pools exhibited by breeding amphibians in plantations is typical for temporary pool breeder. Pools that retain water for longer periods that are not persistent enough for the colonization of aquatic predators facilitate tadpole development (Hartel *et al.*, 2011). Although we did not find amphibians inhabiting plantation habitats in forests, and indeed others have also shown distinct communities (Gillespie *et al.*, 2012; Faruk *et al.*, in press), based on oviposition site choice by temporary pool breeders, there was no selectivity for pools specific to the plantation habitat. If these amphibians were specialized to the oil palm habitat in terms of breeding, we would expect a selection toward variables associated with plantation pools that were distinct from those found in the forest.

With the current data I am unable to make any conclusive argument on whether tadpoles in plantations are habitat specialists or generalists. The rapid developmental time, typical of species found in plantation pools, are indeed useful strategies for development in pools of exposed habitats, but this is an adaptation to temporary pools, and not necessarily a trait specialized to plantations. I am unable to conclusively determine if these

tadpoles have the ability to change their habits in response to a different habitat. Partitioning between feeding guilds is a possible sign of specificity, but it shows a specialization to a particular component of a pool, not necessarily specialization towards a specific habitat type; filter feeders are only able to survive in pools with sufficient particulates to feed on, regardless of the overall habitat type. Therefore, tadpoles of species inhabiting plantations may exhibit specific feeding habits and are well adapted to deal with threats of desiccation, but may not, strictly, be specialist to pools of altered habitats. In my system, both oil palm plantation and forest pools were sufficient to support algae grazers and filter feeders. However, there was an absence of predatory tadpoles in plantation pools, indicating that these pools may not be suitable for tadpoles exhibited such feeding strategies. Nevertheless, the diversity of different feeding strategies may indicate that pools in plantations have the potential to support communities of amphibian tadpole species without strong competition for resources.

Anthropogenically altering landscapes will inevitably lead to rapid changes at the habitat-level, favouring species that are able to utilize features associated with such environments. Based on this study I found that temporary pools in plantations exhibited different characteristics to pools in forests. However, species found in these types of disturbed habitats did not show strong associations with features specific to disturbed habitats. Instead, their preferences were for features commonly associated with a temporary pool breeding species. I can argue that the collection of species do exhibit breeding behaviour specialized for temporary pools, but this trait is not solely restricted to disturbed environments. In terms of management, pools in plantations are variable and some show suitability for sustaining amphibian larvae. Additionally, the feeding guilds of tadpole species found in these altered pools are similar to those found in forest pools, indicating that these pools have nourishment that can support similar forest tadpoles. However, incorporating forest species into plantation habitats may prove difficult as the species currently inhabiting plantations are generally adapted to thrive in such environments and the absence of predatory tadpoles may indicate that, although, herbivorous

and particulate feeders may be able to thrive here, prey items to sustain predators within aquatic systems may be lacking.

Chapter 4

Microhabitat preferences of amphibians in oil palm plantation and secondary forests

4.1 Introduction

Over the past four decades, the human population has doubled to 6.5 billion and is projected to exceed 9 billion by 2050 (Population Reference Bureau, 2006). As the human population grows, so do its demands, resulting in a rapid encroachment and modification of natural habitats (Ragauskas *et al.*, 2006). By modifying the natural habitat, we are directly removing environmental factors of the natural landscape associated with a species or community of species (Morrison *et al.*, 2006). The link between wildlife and its chosen habitat can range from being direct and obvious (e.g. structural modification by mammals) (Jones *et al.*, 1996) to indirect and subtle (e.g changes in soil chemistry via dung deposits) (Batzli, 1994). Additionally, environmental factors within a habitat can be interrelated, for example, the link between canopy cover and soil moisture (Royer *et al.*, 2012). Such relationships can be highly complex, therefore, anthropogenically modifying natural habitats will ultimately affect biodiversity, at times, with negative consequences (Mace & Reynolds, 2001; Foley *et al.*, 2005).

A habitat suitable for a particular organism typically includes important factors for its survival, namely, space, nutrition and shelter, which makes the habitat an important aspect for conservation management

(Morrison *et al.*, 2006). However, within a particular habitat, there are environmental features, typically known as microhabitats, which collectively make up the overall habitat. Although it is important to focus on the overall habitat for conservation efforts, often species have relationships with certain features within the overall habitats that may be equally as important. Generally, a natural habitat exhibits a range of environmental gradients at the microhabitat level, which results in a structurally heterogeneous habitat. The overall habitat, however, is a species or organism specific term; suitability for one species/organism does not reflect general utility for others. As this is true for the habitat level, it is also reflected at the microhabitat level, especially for small bodied organisms. Therefore, a habitat that exhibits a range of different microhabitat, each associated with a different species, would naturally have the opportunity to support a coexistence of many species. By modifying such naturally heterogeneous environments into a largely uniform and homogeneous habitat, these small scale variations would be removed, increasing the threat of biodiversity loss. In addition, species showing preferences to specific aspects of the overall environment would generally also exhibit adaptations to utilize the habitat space, for example, the bright colouration and unusual structures of orchid mantis (*Hymenopus coronatus*) and its association with flowers compared to the brown, dead leaf mantis (*Deroplatys desiccata*), associated with twigs and leaves.

In the early 1990s, a model was proposed showing the relationship between structural heterogeneity and species diversity (Huston, 1994). The model predicts that for species with a relatively small body size and low mobility, strong structural heterogeneity should promote high species diversity. This is because a heterogeneous habitat would contain a wider range of environmental components (microhabitats), promoting coexistence of species that require different, but specific microhabitats. Habitat homogenization would promote homogenization across the community and therefore, be detrimental to the maintenance of diversity (Benton *et al.*, 2003; Smith *et al.*, 2004; Reid *et al.*, 2007). Agricultural conversions have been blamed for simplifying the environment, by replacing areas of high vegetative diversity with a single crop type and removing undergrowth

(Krebs *et al.*, 1999; Vellend *et al.*, 2007). If the aim is to promote diversity through heterogeneity, without conserving specific species, agricultural habitats may be managed to increase structural heterogeneity, for example, through the inclusion of other crop types or forest remnants. Additionally, as different species use specific microhabitats within the overall habitat, managing diversity through habitat heterogeneity within a specified area would need to require information about the microhabitat needs of a particular group or groups of organisms to maintain.

Amphibians are relatively small in body size and often hindered by stretches of unsuitable habitats, such as roads (Porej *et al.*, 2004; Rittenhouse & Semlitsch, 2006; Wolts *et al.*, 2008). The Class Amphibia is also highly diverse in its ecomorphology, with certain species exhibiting adaptations in order to thrive in a certain habitat space (e.g. the extensive webbing seen in rhacophorid species in Asia). Habitat selection and partitioning within this group have been studied in both the tropics and temperate zones, with inconsistent results (Heyer, 1976; Gillespie *et al.*, 2004). Such studies, however, are generally focused on a single species or studied in relatively undisturbed habitats, and were not designed to apply knowledge of microhabitat requirements towards agricultural management. Nonetheless, there is evidence of habitat preferences of adults for various amphibian species were found, for example, species partitioning along streams in Sulawesi (Gillespie *et al.*, 2004). In contrast, a number of studies have failed to show clear habitat preferences, such is the case for *Rana temporaria* in the temperate zone (Grözinger *et al.*, 2012), and *Rana chalconata* in the tropics (Gillespie *et al.*, 2004). Therefore, amphibian species that do not show associations with certain microhabitat features would possibly be able to occupy all the available spaces within the occupied habitat. If this is the case, then the influence of structural heterogeneity on amphibian diversity can depend on the type of species and their associations to the available features of the occupied habitat.

In Chapter 2, I predicted that amphibian species diversity would be lower in oil palm plantations compared to forested habitats. This was not the case. Comparable diversities between forest and plantation sites

sparked the idea that plantations may exhibit a degree of structural heterogeneity comparable to forests. If plantations are heterogeneous habitats for amphibians, then species occupying such a habitat would show associations with specific microhabitat features, have morphological adaptations associated with those features and show partitioning among different species based on variation of microhabitat and ecomorphology. Alternatively, plantations may be structurally homogeneous and the species present have the ability to occupy every microhabitat space within the overall habitat. In this case, amphibians would not exhibit any evidence of partitioning and would exhibit similar ecomorphology.

4.2 Methods

4.2.1 Data collection

Fieldwork was undertaken in the state of Pahang, Peninsular Malaysia, during the summers of 2009 and 2010. Surveys were conducted in three plantation sites (Sungai Mai, Lanchang and Kerdau) and two secondary forest sites (Bukit Rengit and Kuala Lompat). A full description of the study sites including average temperature and rainfall are available in the Method section of Chapter 2. Surveys of each site started around 8pm, when frogs were actively calling, and ended around midnight. Each site was surveyed five times in 2009 and four times in 2010.

Amphibians were searched for in all three 30m X 2m plot types (stream, riparian and terrestrial) (refer to Figure 2.2 for a diagrammatic representation). Riparian and terrestrial plots were marked and searched in accordance with the methodology outlined in Chapter 2. Streams were tagged every 10m for 300m and searched for amphibians using spot lights. Individuals were caught when possible and identified to species. Photographs were also taken for re-identification later. Distinctive morphological features of caught individuals were noted, such as the presence of toe pads and/or webbing. These particular traits were chosen as they show variability across different species and are typically associated with the ecology of each species.

I recorded the substrate each individual was captured on (water, mud/sand, leaf litter, leaf, branch/twig, log or rock), as well as its height above ground measured to the nearest centimeter. Horizontal distances between the individuals location and various microhabitat features were also recorded. For riparian and terrestrial plots I recorded the type of and the distance to the nearest water source as well as the distance to the closest source of cover and/or perch site, e.g. leaf litter pack (a collection of $\geq$5 dried leaves), log/buttress ($\geq$10m diameter at its thickest part), rock ($\geq$0.25m diameter), small tree ($\leq$50m diameter at breast height), large tree (>50m diameter at breast height) and seedlings/undergrowth (foliage $\leq$0.50m in height). I classified water sources based on water permanence (temporary/permanent). Water sources classified as temporary were pools or small, intermittent streams that dried completely at least once per calendar year, while water sources classified as permanent were streams or large ponds with no known drying period. For individuals found in stream plots, I recorded the following: horizontal distance to stream bank, closest log/buttress ($\geq$25m diameter at its thickest part), sand mound ($\geq$0.50m across), leaf litter (a collection of $\geq$5 dried leaves), riffle (moving stretch of water >0.5m in length), pool (stagnant water >0.5m in length), small tree (<50m diameter at breast height) and large tree (>50m diameter at breast height).

Stream, riparian and terrestrial plots were visited during the day to measure the same microhabitat variables, but at random locations. Random locations corresponded to three sets of Cartesian coordinates in a three-dimensional space and generated using a random number table. Random stream locations were chosen based on the three coordinates corresponding to the start of the transect, height above water and distance from bank. I used the outcome of a tossed coin (tails=left; heads=right, when facing upstream) to determine the side of the stream where these locations were situated. Random riparian and terrestrial locations were similarly determined by using randomly generated coordinates, and the numbers corresponded to the distance along the longer side of the plot (y-axis), the shorter side of the plot (x-axis) and height above ground. Microhabitat variables were measured for an equal number of random and

amphibian locations for each plot.

4.2.2 Data analysis

Due to low numbers of amphibians detected in riparian and terrestrial forest plots, data from both plot types were combined into one category called terrestrial frogs. The three most abundant ($\geq$10) species within each plot were chosen for subsequent analysis, with the exception of forest terrestrial plots, where only two species were used. Continuous habitat variables were standardised to z-scores (zero mean and unit variance). Location substrates were converted into integers (0-water, 1-mud/sand, 2-leaf litter, 3-leaf, 4-branch/twig, 5-log and 6-rock) as was type of water source (1-permanent, 2-temporary) and non-stream plot types (1-riparian, 2-terrestrial). These converted variables were treated as factors in subsequent analysis.

Habitat measurements for each species and random location were converted into Euclidean distance matrices. I used the distance matrices to construct corresponding ordination diagrams using Non-metric Multi-Dimensional Scaling (NMDS) using the vegan package in R. The function (*metaMDS*) used to generate distance matrices and construct ordinations uses functions based on Minchin's (1987) recommendations. Two separate analyses were done: 1) comparing microhabitat features between amphibian and random locations, 2) comparing microhabitat features between different amphibian species. Habitat variables were fitted onto corresponding ordinations using the *envfit* function, which also generates p-values to identify degree of fit to the data. I only plotted variables on the final ordinations that had highly significant fit to the data (p-value ≤ 0.05). P-values were used here as individual numbers were small, in contrast to ordination techniques used in Chapter 2.

Permutational multivariate analysis of variance (PerMANOVA) with the same Euclidean distance matrices were used to identify differences in habitat preference. Again, the analysis was done to compare distance matrices between amphibian and random locations, and between different amphibian species. This statistical method is a robust alternative to

parametric MANOVA (multivariate analysis of variance), as it does not assume normality of data or homogeneity of variance. I used the *adonis* function in R for the analysis and F-tests, based on sequential sums of squares from permutations of raw data, to calculate significance.

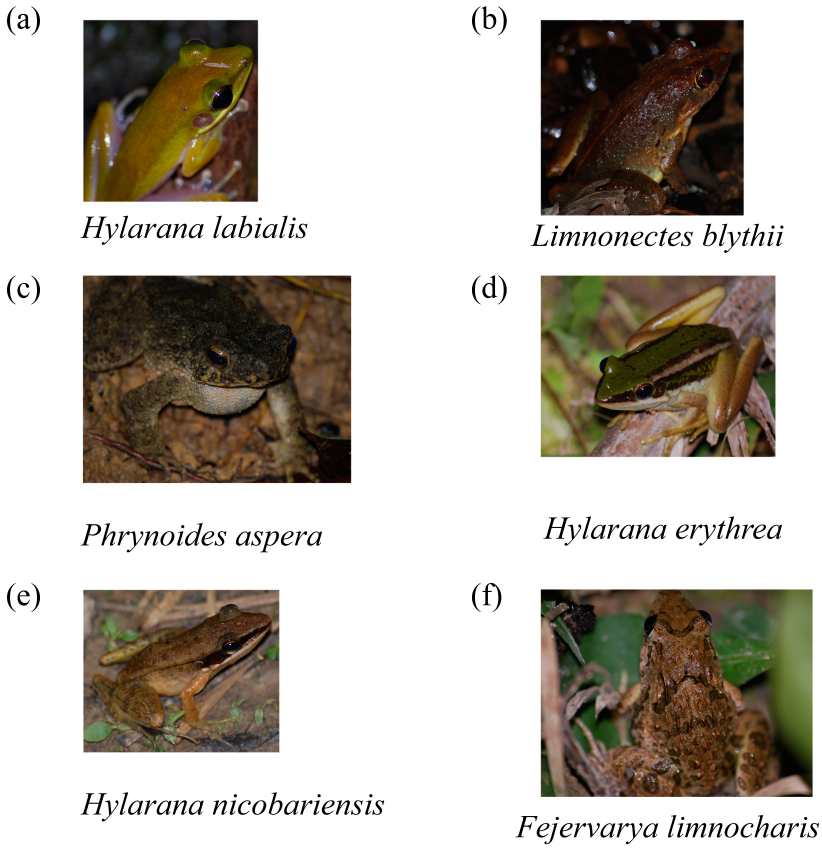
4.3 Results

A total of 12 amphibian species were found in plantation sites and 13 in forest sites throughout the study season, and I detected 302 individual frogs. Abundant species found in forest streams were *Hylarana labialis* (39 individuals found), *Limnonectus blythii* (15) and *Phrynoides aspera* (10) (Figure 4.1a, b and c). In plantation streams, *Hylarana erythrea* (22), *Hylarana nicobariensis* (12) and *Fejervarya limnocharis* (10) were most abundant (Figure 4.1d, e and f). Abundant species found in forest terrestrial plots were *Hylarana labialis* (10) and *Microhyla mantheyi* (10) (Figure 4.2a and b), while in plantation terrestrial plots *Microhyla heymonsi* (53), *Kaloula pulchra* (44) and *Fejervarya limnocharis* (64) were most abundant (Figure 4.2c, d and e).

All amphibians detected along streams did not have full webbing between digits, which is the typical adaptation to being fully aquatic. *Hylarana labialis* had well developed digital discs, indicating a generally arboreal species. *Hylarana erythrea* and *Hylarana nicobariensis* had small, but still distinct, digital discs. *Limnonectes blythii* had moderate webbing on their hind feet without expanded digital discs, indicating that this species is possibly ground dwelling and semi-aquatic. *Phrynoides aspera* and *Fejervarya limnocharis* exhibit similar characteristics with *L. blythii*. In terrestrial plots, individuals from all the Microhylidae group show similar morphological adaptations (e.g. lack of extensive webbing and lack of enlarged toe disks), indicating adaptations to being mainly terrestrial species. Only *Microhyla heymonsi* individuals have moderate webbing, possibly indicating a higher affinity to aquatic environments compared to other microhylids.

NMDS ordinations for amphibian and random locations reached a stable solution at k=2 (where k is the maximum number of dimensions),

Figure 4.1: Amphibian species detected most frequently in forest (a, b and c) and plantation (d, e and f) streams, with full latin names below each corresponding picture



with the exception of *Hylarana erythrea* in plantation streams, where, a stable solution was reached at k=3. Final stress values for all the fitted NMDS configuration of species and random sites indicated adequate fit between dissimilarity matrices and ordination configurations (Table 4.1). Habitat-spaces for two of the forest stream species,*Hylarana labialis* (Figure 4.3a) and *Phrynoides aspera* (Figure 4.3e), were separated from random locations on the first NMDS axis. *H. labialis* was strongly associated with increasing height, distance to rocks, riffles and large trees (Figure 4.3b), in contrast, *P. aspera* showed associations with decreasing height, distance to logs and large trees (Figure 4.3f). The third forest stream

Figure 4.2: Amphibian species detected most frequently in forest (a and b) and plantation (c, d and e) terrestrial zones, with full latin names below each corresponding picture

(a)

Hylarana labialis

(b)

Microhyla mantheyi

(c)

Microhyla heymonsi

(d)

Kaloula pulchra

(e)

Fejervarya limnocharis

Table 4.1: Stress values and number of dimensions (k) generated from constructing Non-metric Multidimensional Scaling ordinations of Euclidean distance matrix of microhabitat features between amphibian location and random location

Species	**Stress**	**k**
Stream (plantation)		
Fejervarya limnocharis	0.101	2
Fejervarya nicobariensis	0.168	2
Hylarana erythrea	0.233	3
Stream (forest)		
Hylarana labialis	0.245	2
Limnonectes blythii	0.172	2
Phrynoides aspera	0.128	2
Terrestrial (plantation)		
Kaloula pulchra	0.138	2
Fejervarya limnocharis	0.179	2
Microhyla heymonsi	0.189	2
Terrestrial (forest)		
Hylarana labialis	0.155	2
Microhyla mantheyi	0.091	2

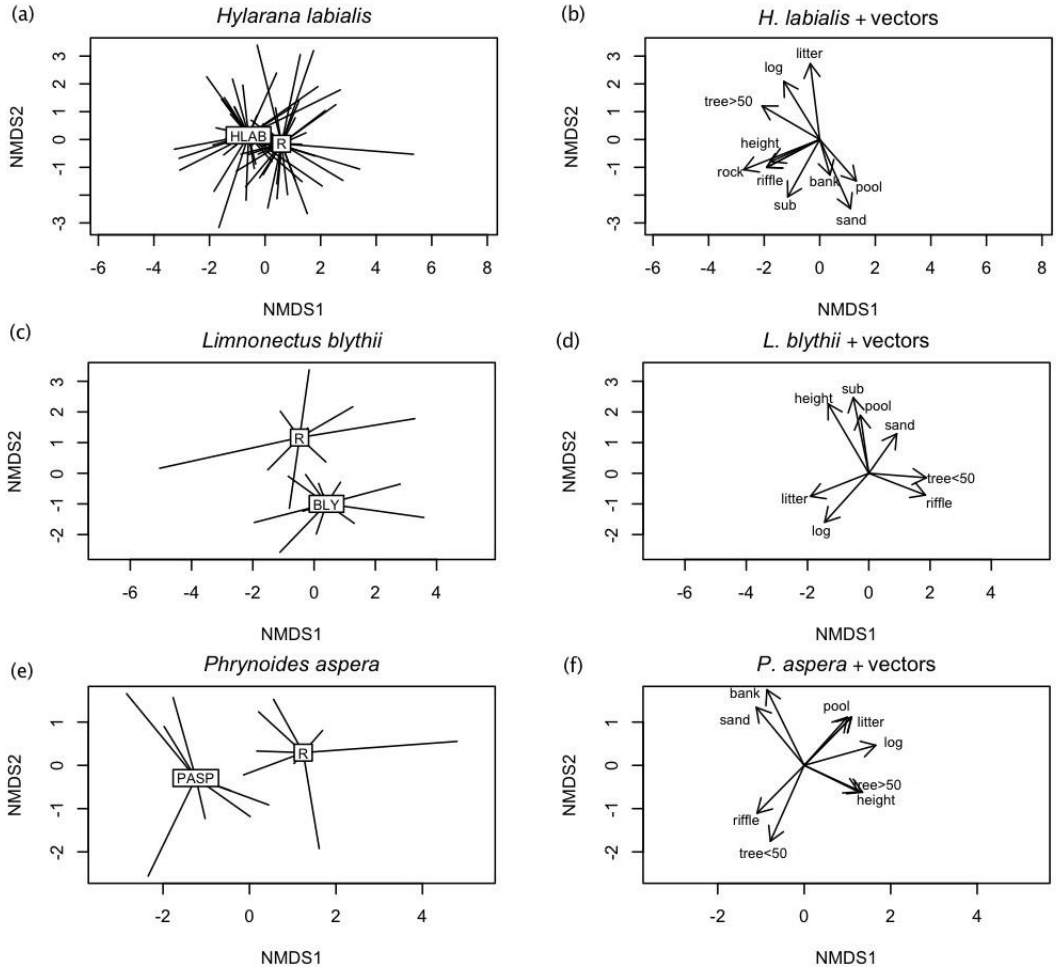
species,*Limnonectes blythii*, was separated from random sites on the second NMDS axis (Figure 4.3c), which represented a gradient of increasing height and pool distance (Figure 4.3d). In plantation streams,*Fejervarya limnocharis* was separated from random sites on the first axis, which represented a gradient of increasing height and decreasing distance to leaf litter (Figure 4.4a and 4.4b). *F. limnocharis* showed associations with decreasing height and increasing distance to leaf litter. Individuals from the two remaining plantation stream species, *Hylarana erythrea* and *Fejervarya nicobariensis*, were separated from their corresponding random locations by having a slight positive association with the second axis and a slight negative association with the first axis (Figure 4.4c and 4.4e). *H. erythrea* individuals were associated with decreasing substrate value and increasing

distance to large trees (second axis), along with decreasing distance from sand mounds and increasing distance from leaf litter (first axis) (Figure 4.4d), while *F. nicobariensis* individuals were associated with decreasing distance to logs (first axis), but increasing distance to leaf litter (second axis) (Figure 4.4f).

In terrestrial forest plots, *Microhyla mantheyi* adults showed habitat associations based on increasing distance from logs, decreasing height and decreasing distance from large trees (Figure 4.5a-b). In contrast, *Hylarana labialis* adults did not show clear separation from all the available habitat space (Figure 4.5c and d). All the species found in plantation terrestrial plots showed associations with certain habitat variables (Figure 4.6a, c and e). Based on the first NMDS axis,*Fejervarya limnocharis* was negatively associated with woody debris (litter and log) and large trees, but positively associated with location substrate. The second axis of the same ordination exhibited associations with distance from rock and seedling (Figure 4.6b). *F. limnocharis* were negatively associated with woody debris and large trees but with a preference to seedlings/undergrowth. *Kaloula pulchra* and *Microhyla heymonsi* both showed an association for being close to the ground, as second axis of the former species and the first axis of the latter species were associated with height measurements. *K. pulchra* individuals were also negatively associated with large trees, while *M. heymonsi* adults preferred were negatively associated with small trees (Figure 4.6d and f). With the exception of *Hylarana labialis* individuals found in terrestrial plots, results from the PerMANOVA indicated that the microhabitat occupied by each species differed significantly from all available habitats (Table 4.4).

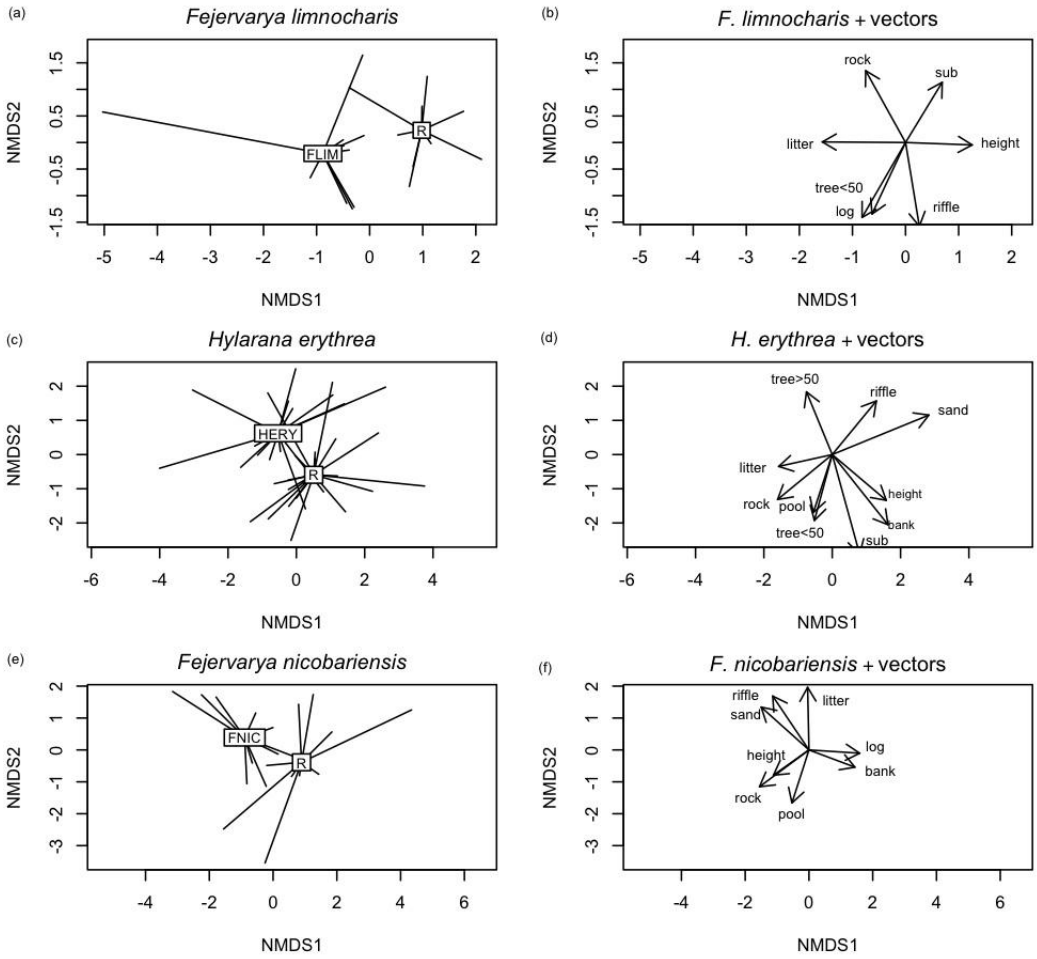
Final stress values for all stream and terrestrial NMDS configurations indicated an adequate fit between dissimilarity matrices and corresponding ordination configurations. Both forest and plantation stream ordinations reached a stable solution with minimum stress at k=2, while ordinations of terrestrial plots reached a stable solution with minimum stress at k=3 (Table 4.3). I found evidence of habitat partitioning between forest stream-dwelling species (Figure 4.7a, Table 4.4). *Hylarana labialis* adults were separated from both *Limnonectes blyhtii* and *Phrynoides aspera* adults

Figure 4.3: Plots of the first and second NMDS axes for amphibian species found in forest streams and randomly selected sites (a, c and e). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object, with random locations labelled as "R". Plots on the right hand side are environmentally fitted vectors for the corresponding species (b, d and f). The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, moving water = riffle, stream bank = bank, sand mound = sand, stagnant water = pool and the type of substrate individual was found = sub.



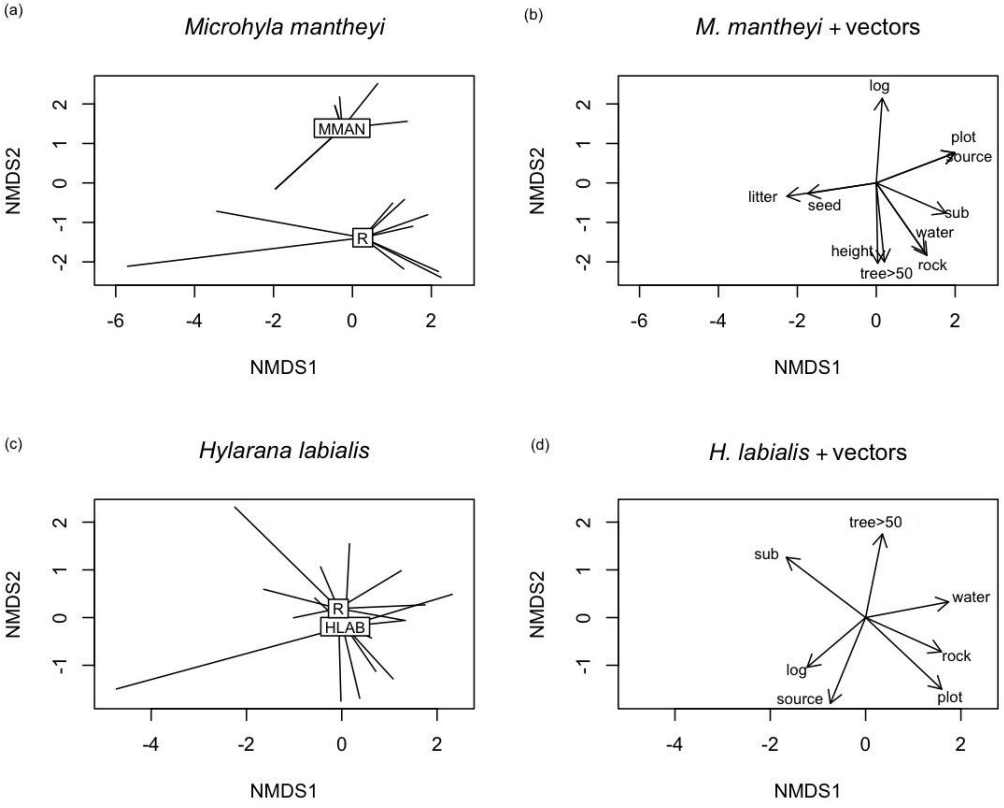
along the first axis (Figure 4.7a), while the remaining stream-dwelling species were separated from one other along the second axis. NMDS 1 was associated with distance from rocks, pools and height above water

Figure 4.4: Plots of the first and second NMDS axes for amphibian species found in plantation streams and randomly selected sites (a, c and e). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object, with random locations labelled as "R". Plots on the right hand side are environmentally fitted vectors for the corresponding species (b, d and f). The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, moving water = riffle, stream bank = bank, sand mound = sand, stagnant water = pool and the type of substrate individual was found = sub.



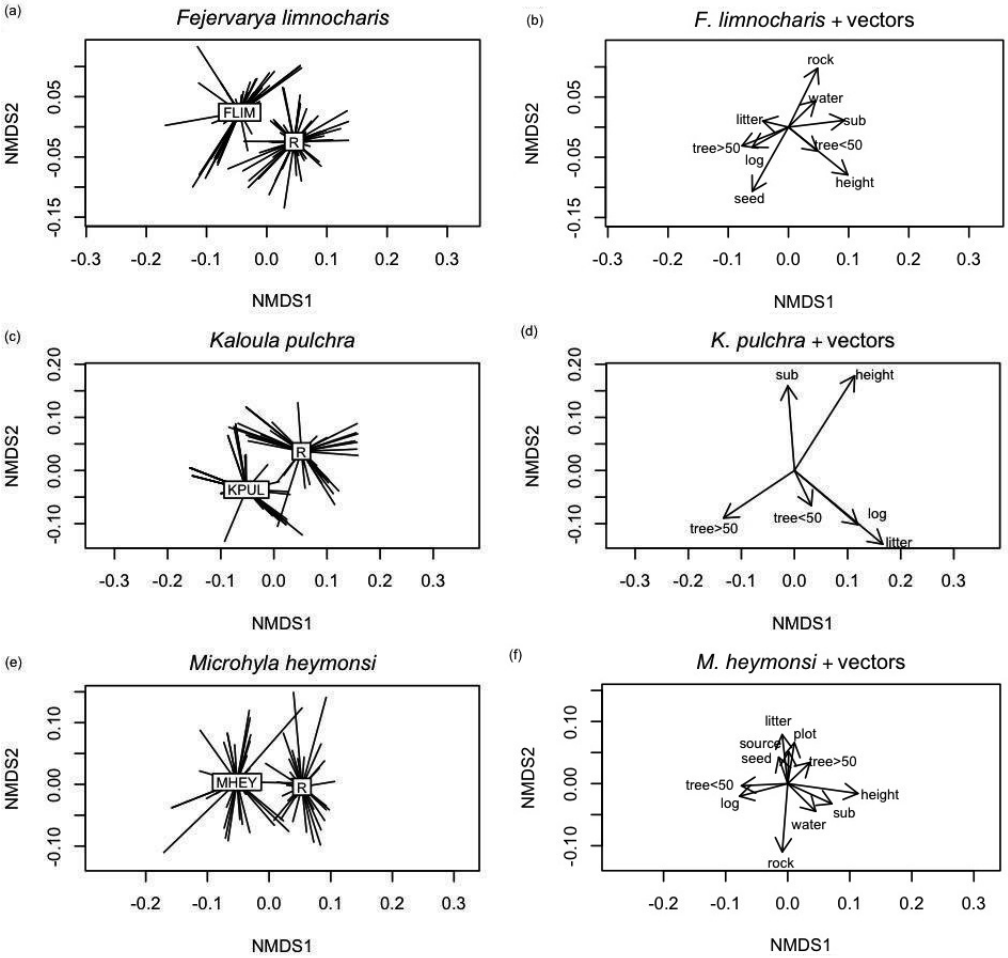
(Figure 4.7b). According to the ordination, *H. labialis* preferred to be higher above water, but close to pools. The separation between *L. blythii* and *P. aspera* adults along the second axis indicated the preference of *P.*

Figure 4.5: Plots of the first and second NMDS axes for amphibian species found in forest terrestrial and randomly selected sites (a and c). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object, with random locations labelled as "R". Plots on the right hand side are environmentally fitted vectors for the corresponding species (b and d). The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, foliage = seed, closest water course = water, the type of substrate individual was found = sub and the type of closest water source = type.



aspera for areas with more leaf litter. Plantation stream-dwelling amphibians also showed significant habitat partitioning (Table 4.4), along the NMDS 1 axis (Figure 4.7c). *Fejervarya nicobariensis* was associated with increasing height and decreasing distance to large trees, in contrast to individuals from the *F. limnocharis* species (Figure 4.7d). There were no strong associations between *Hylarana erythrea* and any variables measured during this study (Figure 4.7c).

Figure 4.6: Plots of the first and second NMDS axes for amphibian species found in plantation terrestrial and randomly selected sites (a, c and e). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object, with random locations labelled as "R". Plots on the right hand side are environmentally fitted vectors for the corresponding species (b, d and f). The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, foliage = seed, closest water course = water, the type of substrate individual was found = sub and the type of closest water source = type.



Forest terrestrial-dwelling amphibians showed significant habitat partitioning (Table 4.4) along the first and second axis (Figure 4.8a). *Hylarana labialis* individuals showed a preference for streams, and located higher

Table 4.2: Results of ADONIS tests, comparing habitats occupied by each amphibian species with randomly selected sample of all available habitat.

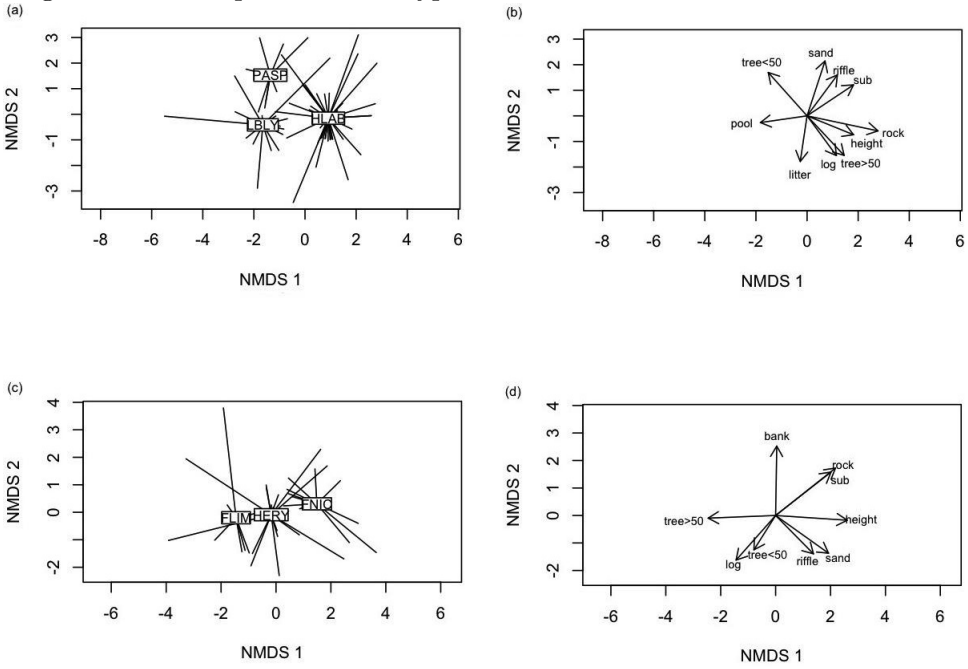
Species VS Random	Df	Pseudo-F	P-values
Forest stream			
Hylarana labialis	73	4.74	0.001
Limnonectes blythii	25	4.26	0.001
Phrynoides aspera	15	2.93	0.001
Plantation stream			
Fejervarya limnocharis	18	5.44	0.001
Hylarana erythrea	40	4.40	0.001
Fejervarya nicobariensis	21	3.02	0.001
Forest terrestrial			
H. labialis	17	1.46	0.16
Microhyla mantheyi	17	6.54	0.001
Plantation terrestrial			
F. limnocharis	116	98.7	0.001
Kaloula pulchra	87	5.51	0.002
Microhyla heymonsi	74	4.18	0.015

Table 4.3: Stress values and number of dimensions (k) generated from constructing Non-metric Multidimensional Scaling ordinations of Euclidean distance matrix of microhabitat features between locations of different amphibian species

Site	**Stress**	**k**
Stream		
Forest	0.178	2
Plantation	0.138	2
Terrestrial		
Forest	0.134	3
Plantation	0.149	3

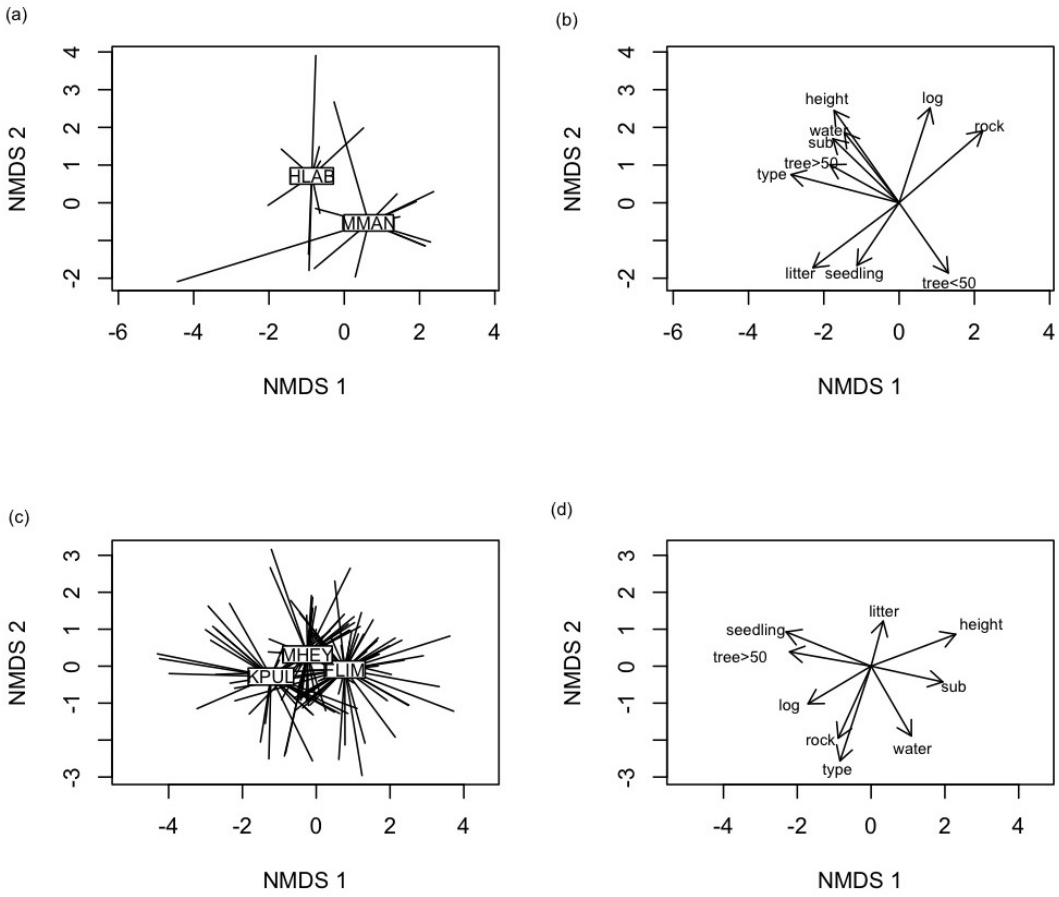
in the habitat space compared to *Microhyla mantheyi*, which showed a higher affinity to temporary pools and was located closer to the ground (Figure 4.8b). Additionally, *H. labialis* individuals were further away from

Figure 4.7: Plots of two dimensional NMDS ordinations of commonly found species in forest (a) and plantation (c) streams, with corresponding microhabitat variables measured for each individual ((b) and (e), respectively). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object. The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, moving water = riffle, stream bank = bank, sand mound = sand, stagnant water = pool and the type of substrate individual was found = sub.



streams, while *M. mantheyi* individuals were found close to pools (Figure 4.8b). Plantation terrestrial-dwelling amphibians also showed significant habitat partitioning (Table 4.4), however, the separation between species was less distinct compared to its forest counterpart (Figure 4.8c). The first axis represented a gradient of increasing height and substrate, but with a negative association with large trees and undergrowth. The species found within these plots were separate along this gradient (Figure 4.8c). *Fejervarya limnocharis* individuals preferred to be higher in the habitat space, compared to the other species within the same habitat. *Microhyla heymonsi* showed an association for temporary pools (water type associated

Figure 4.8: Plots of two dimensional NMDS ordinations of commonly found species in forest (a) and plantation (c) terrestrial plots, with corresponding microhabitat variables measured for each individual ((b) and (e), respectively). Full scientific names for each species and their corresponding abbreviations, labelled within each object, are found in Appendix A.1 of this thesis. Labels represents the centroid for each object. The length and angle of each vector represents the strength and direction of association. Variables measured with stream plots include horizontal distance (cm) to closest leaf litter = litter, log/buttress = log, large trees = tree>50, small trees = tree<50, height above ground = height, rock = rock, foliage = seed, closest water course = water, the type of substrate individual was found = sub and the type of closest water source = type.



with NMDS 2), but not strongly associated with variables represented by the first axis (Figure 4.8d).

Table 4.4: Results of ADONIS tests, comparing habitats partitioning between commonly detected species found per habitat and plot type.

Species VS Species	Df	Pseudo-F	P-values
Forest stream			
Hylarana labialis vs *Limnonectus blythii*	53	10.5	0.001
H. labialis vs *Phrynoides aspera*	46	6.52	0.001
L. blythii vs *P. aspera*	22	3.20	0.001
Plantation stream			
Fejervarya limnocharis vs *Fejervarya nicobariensis*	21	8.43	0.001
F. limnocharis vs *Hylarana erythrea*	28	2.96	0.007
F. nicobariensis vs *H. erythrea*	30	3.22	0.004
Forest terrestrial			
H. labialis vs *Microhyla mantheyi*	20	2.98	0.011
Plantation terrestrial			
F. limnocharis vs *Kaloula pulchra*	90	11.5	0.001
F. limnocharis vs *Microhyla heymonsi*	104	6.44	0.001
K. pulchra vs *M. heymonsi*	73	5.60	0.001

4.4 Discussion

Morphology of species found in both habitats showed characteristics that were associated with the various microhabitat features of the overall habitat. Furthermore, there were similarities in morphological characteristics of amphibian species found in both habitat types, which reflected the similarities of corresponding habitat associations. For example, the morphological similarity between *Hylarana labialis* and *Fejervarya nicobariensis* (i.e. enlarged toe discs) was also reflected in the similarity of their habitat associations (i.e positive correlation with increasing height above ground and trees). Therefore, even though the two habitats are categorically different based on macrohabitat variables, seen in Chapter 2, species in plantations have similar adaptations to those of their forest counterpart, indicating that there may be similar microhabitat features between habitat types. Even though we did not test for evidence of direct replacement, the similarity in ecomorphology and subsequent habitat associations (height and the use of trees as perch sites along streams) indicates the possibility

of species more adapted to disturbances occupying the habitat spaces of less tolerant species that were lost after conversion.

Microhabitat associations and apparent partitioning in my forest site was consistent with other studies of Southeast Asia amphibians (Keller *et al.*, 2009; Gillespie *et al.*, 2005). The only species that lacked association with any of the variable measures were *Hylarana labialis* away from streams. The lack of associations for this species was not, however, indicative of forest terrestrial sites being less structurally heterogeneous, as the other species found in the same habitat space showed associations with specific microhabitat features. In addition, when I compared the microhabitats used by the two forest terrestrial site species, these were separated based on height and small trees. The pattern seen for *H. labialis* possibly reflects breeding preferences, as only the adults found along the streams showed microhabitat associations. Those away from streams were mainly juveniles (A. Faruk, *pers obs.*) and exhibited the ability to utilize all the available habitat space away from streams, as they failed to show any separation from random locations. Differences in habitat utilization based on life-stages emphasizes the 3-dimensional nature of a habitat. A single species may be able to utilize the same habitat space with another during a certain stage, for example, during breeding, but show evidence of partitioning at other times. This was also seen in amphibian species of plantation sites, where each species exhibited associations and partitioning between species, but were known to breed in deep temporary pools based on work done in Chapter 3.

Two of the three species found in plantation streams showed a preference for open areas (away from litter or trees). I am aware that the detection probability in open areas can be substantially higher compared to areas with dense undergrowth (Schmidt, 2003) leading to higher numbers of individuals shown to be associated with characteristics of open areas. However, if there was sampling bias and detection uncertainty, I would expect similar patterns for forest stream amphibians (away from trees or vegetation). Instead, I detected strong associations with vegetation in forest streams. Similarly, species found in terrestrial plots, where I would expect that the effects of detection uncertainty would be strongest,

show strong correlations with large trees and undergrowth. It is possible that open areas provide more opportunities to find mates or prey, however, this association with open spaces is most probably a byproduct of plantation management. Due to the possibility of the stream bank collapsing, palms are not planted close to streams and trees and litter are not typically found along plantation streams (A. Faruk, *per. obs.*). As a result, large stretches of streams within plantation sites are open areas with little or no woody vegetation along streams. The lack of structural complexity was not universal for plantation stream plots, as *Fejervarya nicobariensis* was associated with vegetation along plantation streams and was partitioned from the other two along a height gradient. Although found in other disturbed habitats (Onn *et al.*, 2010), *Fejervarya nicobariensis* was found more commonly in the plantation that had a degree of riparian vegetation along its streams, illustrating how structural complexity may vary within, as well as among habitats.

Although species along plantation streams were found to associate with open areas, the same could not be said for species found in plantation terrestrial plots. Here, the species I studied were partitioned along the axis associated with foliage and trees (in this case, palms). The only other study of oil palm plantations and amphibian diversity failed to detect any species from the Microhylidae family (Gillespie *et al.*, 2012). However, microhylids, a group of amphibians typically associated with leaf litter, were present in plantations studied here, exhibited habitat partitioning and readily bred within the available temporary pools (refer to Chapter 3). A possible explanation for the disparity between this and the Gillespie study could be down to the absence of the particular species of microhylids commonly found in my system in Borneo, but there could also be differences in the structure or management among plantations between our two studies. Indeed, studies have shown how structural differences between different altered habitats can affect species richness and individual densities (Vallan, 2002).

In summary, amphibians in both habitats showed morphological adaptations that corresponded to their chosen microhabitat features, which can be similar even when the overall habitats are starkly different. Although

terrestrial areas within the plantations studied hinted at some degree of structural heterogeneity, species mainly associated with open areas seen along plantation streams indicates a growing concern about heterogeneity along streams. In order to increase diversity, especially of small bodied organisms, subtle details such as the incorporation of woody vegetation and leaf litter cover could provide sufficient structural complexity within plantation sites. Additionally, for each organism type, managed habitats will still need to fulfill the requirements for sustaining a population, i.e., providing shelter, nourishment, and sufficient space.

Chapter 5

Effects of habitat conversion on amphibian health

5.1 Introduction

As demands from the ever growing human population increases, habitat loss, which constitutes habitat conversion as well as habitat destruction, is also increasing (Wanger *et al.*, 2009). Changes in the structure of natural landscapes are not only detrimental on species dependent on such natural resources (Todd & Rothermel, 2006; Devictor *et al.*, 2008), but can also lead to changes in important processes, including those between hosts and parasites (Walsh *et al.*, 1993; McKenzie, 2007). In light of such rapid changes to the natural environment, and the widespread emergence of human and wildlife diseases, determining how habitat alteration affects host-parasite interactions are becoming increasingly important (Koprivnikar *et al.*, 2012). Habitat loss, infection and disease are factors linked with wild population declines and can act synergistically, but not all infections result in measurable pathology. For example, infection with macroparasites (e.g. helminths and certain arthropods) typically exhibit intensity-dependent pathology, however, the impacts on the individual host are not straightforward, and therefore, the effects on the overall host population can be hard to predict (Albon *et al.*, 2002; Holmstead *et al.*, 2005). In contrast, certain microparasites (e.g. viruses, fungi, bacteria, and protists) can have direct, detrimental effects on the infected host, which in extreme cases lead to high mortality rates within the overall population (Gray *et al.*, 2009; Kilpatrick *et al.*, 2010).

The impact of habitat loss on natural wildlife populations range from severe population declines to subtle changes in abundance (Struebig *et al.*, 2008; Turner & Foster, 2008; Wanger *et al.*, 2010; Gillespie *et al.*, 2012). Therefore, the resulting effects of habitat change on parasite infection may also be as complex, due to the intimate link between parasites and their hosts (Bush *et al.*, 2001). Additionally, different transmission modes (i.e. the presence or absence of different hosts) exhibited by parasites can further complicate matters. For example, many microparasites that are directly transmitted between hosts exhibit a density-dependent transmission, which can eventually be eliminated in small and scattered host populations (Peterson *et al.*, 1998). In contrast, macroparasites and infections (typically sexually transmitted or vector-bourne diseases) exhibiting frequency dependent transmissions are not usually affected by population size/density, but instead, by the proportion of available hosts, so in theory, should be able to persist in small host populations (Lockhart *et al.*, 1996; Antonovics *et al.*, 1995; Ryder *et al.*, 2007). As well as having an affect on host population size, habitat alteration can also lead to substantial changes in host species richness and/or community composition (Struebig *et al.*, 2008; Wanger *et al.*, 2010; Harrisson *et al.*, 2012). Changes in host species richness can have an effect on host-parasite dynamics as most pathogens are known to cross the species barrier (Woolhouse *et al.*, 2001). A number of studies have shown that the inclusion of a shared host can be beneficial to either one or both hosts as it decreases the proportion of infected individuals (Ostfeld & Keesing, 2000; Dobson, 2004). Having multiple hosts may, alternatively, increase the likelihood of disease-induced extinctions of the host species through the presence of reservoirs hosts (de Castro & Bolker, 2005). Therefore, host species richness can influence parasite dynamics by diluting the effects of infection or sustaining the parasite, driving the host species to extinction. Shifting the natural balance of a particular host community can increase the threat of transmission from a resistant host species to a vulnerable host species (Patz *et al.*, 2000; LoGuidice *et al.*, 2003). In addition, changes in the host community can also change the population dynamics of parasites with complex life cycles through the addition or removal of intermediate or final hosts. Therefore,

the impact of habitat alteration on host-parasite dynamics can be complex and ambiguous.

Amphibians are themselves predators and also serve as prey to larger predators therefore they have the potential to act as intermediate and/or definite hosts to a range of parasite species. Individual amphibians have also shown to support a variety of parasite species at any one time, with several thousand individual parasites being reported in some individuals (Sutherland, 2005; Schotthoefer *et al.*, 2011). Altering natural habitats has been shown to affect amphibians (Gardner *et al.*, 2007b; Ofori-Boateng *et al.*, 2012; Gillespie *et al.*, 2012), but the patterns are not straightforward. Changes in community composition have been reported in all cases, but some studies also report a substantial reduction in species richness and/or abundance (Gardner *et al.*, 2007b; Gillespie *et al.*, 2012), whereas others do not (Faruk *et al.*, in press). Additionally, the distribution of amphibians within the altered habitat may not be uniform, with certain areas being made more unsuitable for amphibians than others (Faruk *et al.*, in press). Due to the relationship between abundance and diversity of host and its parasite, the patterns of infection of amphibians within a rapidly changing landscape may be distinctly different from those found in natural populations and not necessarily uniform across the altered habitat.

In Southeast Asia, habitat loss is mainly driven by agriculture, more specifically, oil palm cultivation (Donald, 2004; Koh, 2007; Koh & Wilcove, 2008). The value of this commodity to Southeast Asian countries is obvious, as it provides stable income, sustains valuable infrastructure and improves the overall economy (Koh & Wilcove, 2008). However, it has been shown to have a negative effect on local biodiversity (Chung *et al.*, 2000; Brühl & Eltz, 2010; Gillespie *et al.*, 2012). To date, the majority of host-parasite studies based in oil palm plantations are generally directed towards oil palm pest management or human vector-bourne diseases, such as malaria or dengue fever (Chang *et al.*, 1997; Pluess *et al.*, 2009; Ntsefong *et al.*, 2012; Mahadi *et al.*, 2012). Although such studies are important in maintaining high yields and the health of those working within and around plantations, the effects this commodity has on natural ecological relationships, such as host-parasite systems, are mostly unknown. Relationships

between parasite infection and agriculture are known from other habitats where certain agricultural practices (e.g. eutrophication, pesticide use, etc) have been found to affect amphibian infection from parasites such as trematodes and digeneans (Kiesecker, 2002; McKenzie, 2007; Rohr *et al.*, 2008). Amphibians seem to be an ideal candidate for exploring this question, as they are found in both disturbed and undisturbed habitats, have shown to be affected by agricultural practices listed above and hosts to a range of parasites with direct and indirect life-cycles (McKenzie, 2007). In addition to identifying the effects of changing parasite patterns on amphibian hosts, the complex life cycles of many parasites can make them potential bioindicators for environmental changes (Hudson *et al.*, 2006; Koprivnikar *et al.*, 2012).

In this study I compare parasite prevalence, intensity, frequency distribution and community composition in amphibian hosts between forest and plantation sites for two microparasite species and a community of macroparasites. The microparasites I studied were the highly virulent fungus *Batrachochytrium dendrobatidis* (*Bd*) and the gut fungus *Basidiobolus ranarum*. *Bd* has been linked to species extinctions and declines of natural amphibian populations (Garner *et al.*, 2005; Pounds *et al.*, 2006), but studies focusing on Asian amphibian have yet to show evidence of extensive population declines or morbidity associated with infection, despite it being detected in nearby countries (Kusrini *et al.*, 2008). *Bd* typically infects the keratinized skin of amphibians and is a generalist amphibian pathogen. This particular fungus causes mortalities directly by affecting osmoregulation leading to cardiac arrest (Voyles *et al.*, 2009), or indirectly by deterioration of the hosts body condition and hinder feeding (Berger *et al.*, 2005). Host-pathogen dynamics for this particular parasite is also largely environmentally dependent (Piotrowski *et al.*, 2004; Kriger *et al.*, 2007; Voyles *et al.*, 2009). There have not been any studies that link *Bd* emergence and agriculture, however, infections have been detected in frogs farmed for food and associated with the amphibian trade (Mazzoni *et al.*, 2003; Schloegel *et al.*, 2009).

B. ranarum is not widely researched, despite it often being prevalent in the environment and, on occasion, infecting humans (Henk & Fisher,

2012). It is not known to be a strict amphibian pathogen, but possibly a pathogen of invertebrates, thus, its existence within amphibian guts is probably due to ingestion of infected prey or passive ingestion of soil (Henk & Fisher, 2012). Additionally, *B. ranarum*'s ability to infect humans and herpetofauna makes it an important pathogen to monitor in terms of transmission to those working and living within plantations (Joe *et al.*, 1956). As both *B. ranarum* and *Bd* are known host generalists, I would hypothesize that if present within my system, they would infect more than one amphibian species. In terms of *Bd*, I would predict a low prevalence in forest hosts, as the only study that detected *Bd* within their sample of forest amphibian species found a 4-14% prevalence across four families of which over 100 individuals were sampled (Savage *et al.*, 2011). The increase in human movements between and within plantation sites would increase the probability of plantation amphibians being infected by *Bd*, which make me predict that prevalence within plantations would be higher than that of forest hosts. *B. ranarum*, in contrast, would show a higher prevalence and intensity in forest amphibians, as its mode of transmission involves insects and past studies on invertebrate diversity and abundance within oil palm plantations revealed a reduced invertebrate abundance compared to forested habitats (Turner & Foster, 2008).

The macroparasites I studied were endoparasitic nematodes, a phylum with approximately 5, 800 parasitic species (Bush *et al.*, 2001). Nematodes exhibit both direct and indirect life-cycles, the latter needing the presence of one or more intermediate hosts. Those with direct life-cycles typically infect their amphibian hosts either through oral or percutaneous transmission. Additionally, nematode parasites may use amphibians as paratenic hosts, where no development takes place, or as intermediate hosts, where development does take place and the host is obligatory for the parasites life cycle (Bush *et al.*, 2001). I predict that patterns of prevalence and intensity of nematode parasites with indirect life-cycles should be similar to *B. ranarum* between the two habitats, with lower parasitism in plantations due to lowered transmission. Nematodes with direct life-cycles, however, should show similar patterns of infection between forest and plantation sites, as abundance and species richness of amphibians were comparable

between habitat types (Chapter 2). In terms of parasite diversity, I would expect a higher diversity in forest sites, as there should be a greater range of hosts within such systems compared to plantation sites. In addition to this, I would also expect a distinct parasite community in each habitat type, as reflected in the distinct host communities between forest and plantation sites.

5.2 Method

Data collection

Study site descriptions and amphibian survey methodology are outlined in the Method section of Chapter 2. I originally planned to collect 40 individuals of the same amphibian species in both oil palm plantation and forest sites. However, due to the distinct community composition between habitat types (Chapter 2), I decided to concentrate on four separate species (two from forest and two from plantation). Species chosen for this comparison were from the same family with similar habitat preferences (1 stream and 1 leaf litter species for each habitat).

At point of capture, I took superficial skin swabs for all individuals using the standard swabbing protocol set out by Hyatt et al (2007). Fresh sterile gloves were used for each individual to avoid cross contamination. Swabs were refrigerated and transported back to the Institute of Zoology laboratory for quantitative-PCR (qPCR) following the techniques described by Boyle et al (2004). After swabbing frogs were placed in individual plastic bags, each bag being labelled with the date of capture, placed in a cooler box and transported to the University Malaya laboratory for further processing. Most frogs were processed within 24 to 48 hours. Once at the laboratory, individuals were housed individually in (30x19x20) cm plastic vivariums. Faecal samples were collected using a sterile micro spoon and placed in a sample pot filled with distilled water. Each pot was labeled with the source amphibian species name, location of capture and date of capture. Labeled pots were sent to Dr Daniel Henk of Imperial College London for the isolation and culturing of *Basidiobolus*

ranarum.

After faecal sample collection, frogs were euthanized by firstly sedating with 0.1% diluted MS-222, after which 0.2ml MS-222 (250mg/l) was injected into the body cavity using a fine gauge 1.0ml insulin needle. After death was confirmed I determined the sex of each individual and took several morphological measurements (snout-vent length, crus length, length of longest toe, thigh length) using a Vernier caliper to the nearest 0.1mm. Euthanized individuals were then inspected for skin lesions or abnormal growths. The viscera was carefully dissected out of the body cavity and placed in a petri dish filled with a 10% dilution of saline solution. The tongue, body cavity and leg muscles were inspected using a stereo microscope and I extracted any nematodes I detected. The throat, heart, lungs, stomach, liver, large and small intestine and duodenum were all dissected and examined for the presence of nematodes using a pair of soft tip tweezers under a stereo microscope.

Nematodes collected from each individual host were initially categorized as small (<5mm) and large (>5mm), separated based on their location within the host and were stored in 70% ethanol solution for genus identification at a later date. Before identifying each nematode, I placed those classified as small directly on a drop of pure glycerol on a microscope slide and left for 20 minutes to clear their outer cuticle. Large nematodes were placed in a solution of creosote for 10 minutes to clear their cuticle and each one transferred onto a drop of glycerol on a microscope slide. Each slide was placed under a light microscope and a collection of keys compiled and edited by Anderson and colleagues (2009) were used to identify nematodes to genus level or as close to genus level as possible. Magnification of the light microscope for nematode identification varied depending on the size of each nematode (63x, 250x or 400x).

Data analysis

All statistical analyses for this chapter were carried out using R (R Developmental Team, 2012). Quantitative descriptors for amphibian parasites

were used based on definitions provided by Bush et al (1997) and recommendations by Rózsa et al (2000). As the *Basidiobolus* dataset was in the form of absence/presence, only prevalence was reported. Observed prevalence was calculated for both *Basidiobolus* and nematode datasets. Prevalence comparisons between forest and plantation hosts were done separately for *Basidiobolus* and nematode datasets, using Fishers exact test. Mean intensity (the arithmetic mean of individual parasites per *infected* host) for each nematode genus was calculated per species and habitat type. I used a measure of intensity, as opposed to abundance (number of individual parasite per *examined* host), as intensity is not strongly influenced by sample size, varies independently from prevalence and tends to be less skewed than abundance distributions, allowing for calculations of confidence intervals to be more precise and informative (Rózsa *et al.*, 2000). Direct comparisons between forest and plantation nematode intensities were done by bootstrapping nematode intensities per habitat type (1999 bootstrap replicates) and performing a t-test. Confidence intervals for prevalence and intensity were calculated using the adjusted bootstrap percentile (BCa), a form of nonparametric bootstrap confidence interval method, which deals with small sample sizes and non-normal distributions (Fox, 2002). For each morphological characteristic, I compared variances between forest and plantation amphibian hosts using F-tests. The mean morphological differences were compared for each species using the Welch two sample t-test, which takes into account samples with unequal variances.

Frequency distributions of nematodes were generated for each host species. A corrected moment estimation of k (derived from the negative binomial distribution models for each host species) was used to measure the degree of parasite aggregation, a method that has shown to vary least with mean parasite load and sample size (Gregory & Woolhouse, 1993). A large value of k (typically >10) would indicate that the distribution converges to a Poisson or random distribution. As k gets smaller, the parasite distribution becomes more aggregated (as variance becomes greater than the mean), and highly aggregated distributions typically have values of k=1 (Wilson *et al.*, 2002).

To identify the effects of habitat, host type and body size on parasite prevalence and intensity, generalized linear models (GLMs) were used. I generated models with binomial errors for prevalence data. Zero-truncated generalized linear models were generated with the intensity models, with either poisson errors or negative binomial errors if the model showed overdispersion (Venables & Ripley, 2002, R package *MASS*) for intensity data. Before model construction, I looked for correlations between morphological measurements (snout-vent length, crus length, toe length, thigh length) and used a principal component analysis (PCA) to identify the variable associated with the first principal component to be used in both models. I used zero-truncated GLMs for the intensity dataset because this dataset does not include zero counts. Parameter estimates between a truncated and non-truncated negative binomial can vary substantially, especially when the majority of the data has values close to zero (intensities of 1 to 5 nematodes). As 70% of the infected hosts had fewer than 5 nematode counts, I decided to initially compare the truncated (Yee, 2012, R package VGAM) and non-truncated models. Comparisons between the two parameter estimates showed no large disparities between the two methods (refer to Appendix G.1). As different hosts were collected for forest and plantation sites, I decided to use host family (Microhylidae and Dicroglossidae) as the explanatory variable for both the prevalence and intensity models as opposed to host species. This would allow me to identify a host effect without being confounded by habitat effects. The initial models (prevalence and intensity) included habitat type, host sex, host family, host snout-vent length and two-way interactions for all variables. Similar models were used to identify effects of each explanatory variable on prevalence and intensity of parasites typically exhibiting direct and indirect life-cycles. For the prevalence models, explanatory variables were dropped sequentially without replacement and I compared models using a Chi-square test within the *anova()* function in R to obtain the minimal adequate model (Crawley, 2007). Intensity models were selected by comparing AIC values of each model after dropping explanatory variables, also without replacement. In this case, interaction terms were dropped from the model first and left out if AIC values did not increase more than 2

units.

Nematode diversity for each host and habitat type was calculated using the Brilluoins diversity index, as it takes into account biased sampling. As I only collected a subset of hosts within each habitat type (forest=2 host species, plantation=2 host species), the resulting parasites collected would be biased towards those associated with the host species collected (Dyke, 2008). Brillouins evenness index was used to calculate evenness for habitat and host type. Finally, differences in community composition between habitat and host type were analyzed using a permutational multivariate analysis of variance (PerMANOVA). Before the PerMANOVA analysis, the datasets were converted into a distance matrix using a variant of Morisita distance index (Horn-Morisita), which is typically used for genuine count data. The same matrix used for the PerMANOVA was then used to generate a Non-metric Multidimensional Scaling (NMDS) ordination diagram for further illustration. All functions for this analysis can be found in the *vegan* package (Oksanen *et al.*, 2012).

5.3 Results

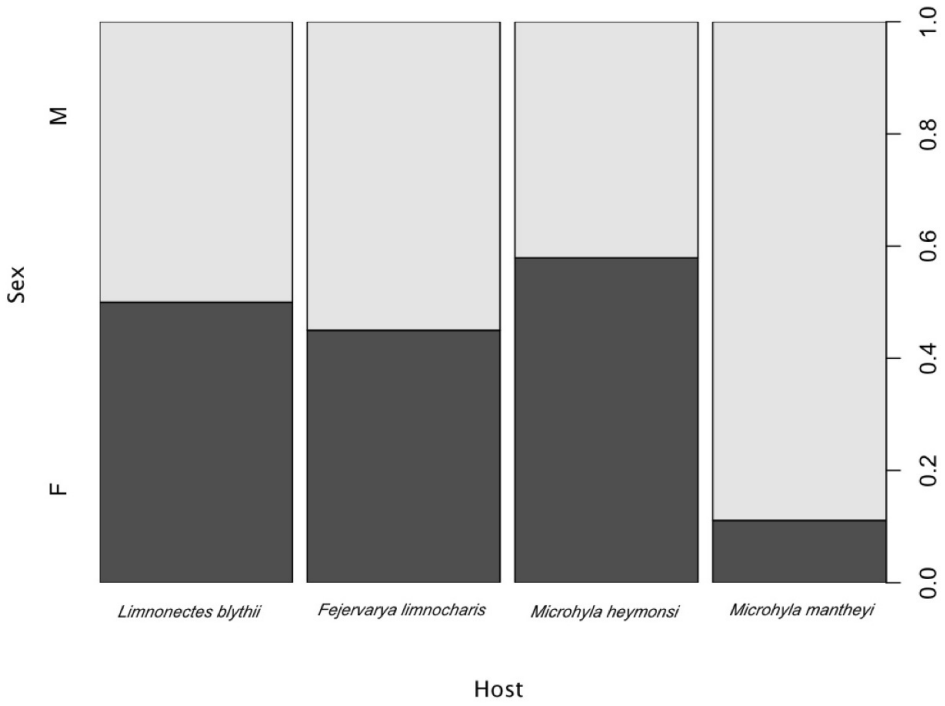
The host species collected for parasite analysis were *Limnonectes blythii* (Dicroglossidae; total number of individuals = 20) and *Microhyla mantheyi* (Microhylidae; 18) from forest sites and *Fejervarya limnocharis* (Dicroglossidae; 20) and *Microhyla heymonsi* (Microhylidae; 21) from plantation sites. The two dicroglossid species are found in streams and riparian areas of their respective habitat types, while the two microhylid species were usually found amongst leaf litter away from permanent streams. All of the species collected exhibited a 1:1 sex ratio, with one exception; a higher percentage of males were present in the *M. matheyi* sample (Figure 5.1).

I failed to detect the presence of *Bd* from either forest or plantation samples using qPCR techniques. We found 4 separate clades of *B. ranarum* within my samples and one undescribed species. On average, 61.3% of faecal samples contained *B. ranarum* and Clade 4 was most prevalent overall. This was also the most prevalent clade in plantation

Table 5.1: Observed percentage prevalence of endoparasites infecting amphibian species oil palm plantation and forest sites. Abbreviations correspond to Figure 5.10 in this chapter. Confidence interval (CI) was calculated using a nonparametic bootstrap method (see text). Table also includes the typical life-cycle for each nematode genus: I=indirect (requiring intermediate host), D=direct (through oral or percutaneous transmission without the need for intermediate host)

Parasite	Abbreviation	Total Observed	CI	Forest	CI	Plantation	CI
Basidiobolus	BAS	62.5	*(38.7, 74.2)*	62.5	*(31.3, 82.5)*	62.5	*(31.3, 81.3)*
Clade 1	B1	12.5	*(3.12, 21.9)*	12.5	*(0.00, 31.3)*	12.5	*(0.00, 25.0)*
Clade 2	B2	15.6	*(3.12, 28.1)*	25.0	*(1.44, 43.8)*	6.25	*(0.00, 18.8)*
Clade 3	B3	9.37	*(0.00, 18.8)*	18.8	*(0.00, 37.5)*	0.00	*(0.00, 0.00)*
Clade 4	B4	21.9	*(6.25, 34.4)*	6.25	*(0.00, 18.8)*	37.5	*(11.4, 56.3)*
Unknown species	BU	3.13	*(0.00, 9.38)*	0.00	*(0.00, 0.00)*	6.25	*(0.00, 18.8)*
Nematode	NEM	83.5	*(72.2, 89.9)*	92.1	*(71.2, 97.4)*	75.6	*(58.5, 85.4)*
Abbreviata (I)	AB	6.33	*(1.27, 11.4)*	10.5	*(2.63, 21.1)*	2.44	*(0.00, 7.32)*
Amplicaecum (I)	AM	21.5	*(12.7, 30.4)*	39.5	*(23.7, 52.6)*	4.88	*(0.00, 12.2)*
Chitwoodchabaudia (I)	CHIT	3.80	*(0.00, 8.86)*	5.26	*(0.00, 13.2)*	7.32	*(0.00, 22.0)*
Maxvachonia (D)	MAX	36.7	*(25.3, 46.8)*	31.6	*(15.8, 44.7)*	41.5	*(26.8, 56.1)*
Neoxysomatium (D)	NEO	2.53	*(0.00, 6.33)*	5.26	*(0.00, 13.2)*	0.00	*(0.00, 0.00)*
Omeia (I)	OM	6.33	*(1.27, 11.4)*	7.89	*(0.00, 15.8)*	4.88	*(0.00, 12.2)*
Oxysomatium (D)	OXY	12.7	*(5.06, 20.3)*	15.8	*(5.26, 26.3)*	9.76	*(2.44, 19.5)*
Rhabdias (D)	RHAB	6.33	*(1.27, 11.4)*	0.00	*(0.00, 0.00)*	12.2	*(2.44, 22.0)*
Waltonella (I)	WALT	1.27	*(0.00, 3.80)*	2.63	*(0.00, 7.89)*	0.00	*(0.00, 0.00)*

Figure 5.1: Sex ratio of amphibians collected



hosts, whereas Clade 2 was the most prevalent in forest hosts. I only found Clade 3 in forest hosts and plantation amphibians were infected by the unknown species (Table 5.1). There were no significant differences in the proportion of individuals infected by *B. ranarum* between forest and plantation amphibians (Fisher exact test: p-value=0.695).

A total of 254 nematodes were extracted during the study, originating from 9 genera that occur in 7 families. On average, 83.5% of amphibians sampled were infected with nematodes. The nematodes were classified as having direct or indirect life cycles on the basis of presence or absence of adults with eggs and later confirmed by past literature. Parasite genera that are typically known to exhibit a direct life-cycle are *Rhabdias* (Baker, 1979) and *Oxysomatium* (Anderson, 2001). The life-cycles of *Maxvachonia* and *Neoxysomatium* are unknown, but based on the characteristics of

their eggs, they are thought to have similar life-cycles to *Oxysomatium* (Anderson, 2001). For all these directly transmitted parasite genera, I found individuals bearing eggs within all the infected hosts. *Chitwoodchabaudia*, *Omeia*, *Abbreviata*, *Amplicaecum* and *Waltonella* are parasites typically exhibiting indirect life-cycles.

Parasites from the genus *Maxvachonia* were most prevalent, followed by the genus *Amplicaecum*. There was no difference in overall nematode prevalence between habitat types (Fisher exact test: p-value=0.991). As with prevalence, *Maxvachonia* followed by *Amplicaecum* showed the highest mean intensities compared to the other nematode genera overall. *Maxvachonia* was more abundant in infected forest hosts, while *Amplicaecum* was more abundant in infected plantation hosts (Table 5.2). Bootstrapped t-test results showed a significant difference in intensity between habitat types (bootstrap t-test; $t_{(35.9)}$=2.6172, p=0.008), with forest amphibians having higher mean parasite intensity compared to plantation amphibians (Table 5.2).

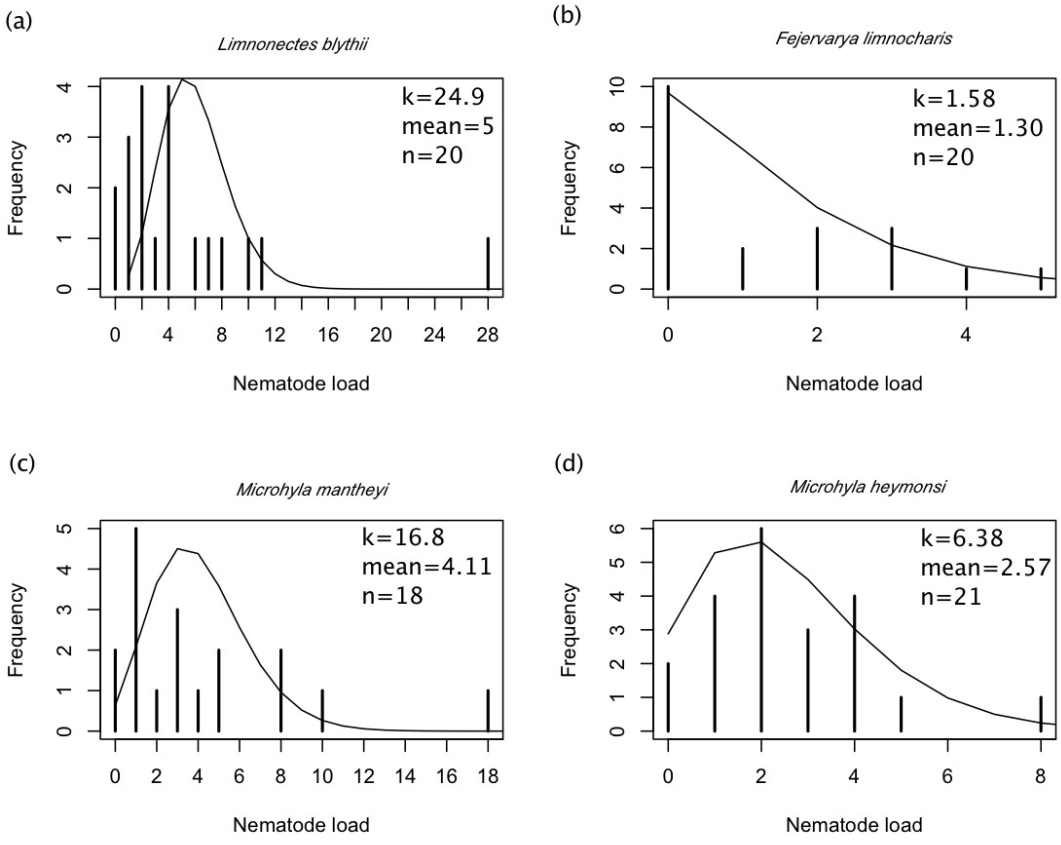
Nematode infections were more aggregated in plantation hosts compared to their forest counterparts. Nematode distributions in *Fejervarya limnocharis* and *M. heymonsi* gave a corrected moment estimate of $k < 10$, whereas parasites in *Limnonectes blythii* and *Microhyla mantheyi* had a distribution that was close to random, with corrected moment estimates of $k > 10$ (Figure 5.2).

Snout-vent length did not differ significantly between the two microhylid or the two dicroglossid species, however, variances did differ significantly between host families. The dicroglossid species from the forest, *Limnonectus blythii*, was significantly more variable in snout-vent length compared to the plantation dicroglossid species, *Fejervarya limnocharis* (F-test: $F_{(19,19)}$=2.78, *p*-value=0.03). In contrast, the microhylid species showed the opposite pattern, with the plantation microhylid, *Microhyla heymonsi* having a more variable snout-vent length than its forest counterpart, *M. mantheyi* (F-test: $F_{(16,20)}$=0.293, p-value=0.02). Forest species had, overall, longer legs than plantation species (Figure 5.3b-c). Variations in thigh length were significantly different between habitat types (Dicroglossids: F-test: $F_{(19,19)}$= 2.71, p-value= 0.04; Microhylids: F-test:

Table 5.2: The mean intensity of nematode genus found in the overall sample, forest and plantation hosts. Confidence interval (CI) was calculated using a nonparametic bootstrap method (see text).

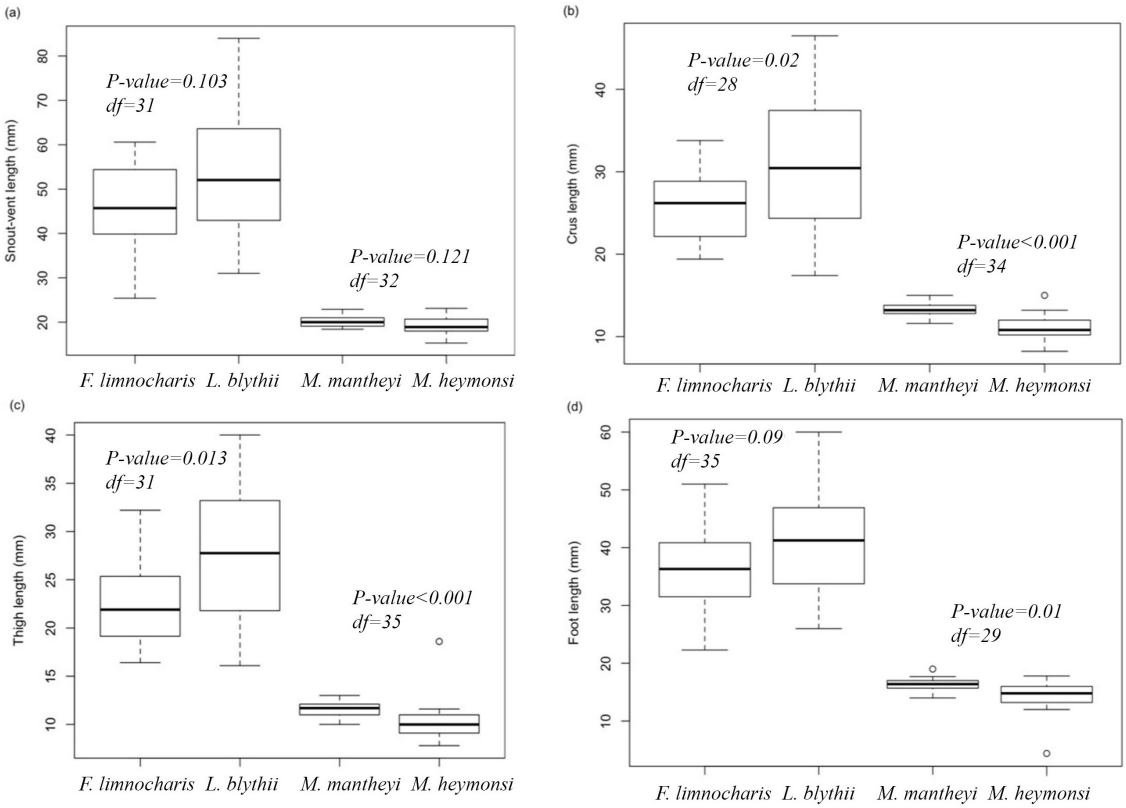
Parasite	Mean overall intensity	CI	Forest	CI	Plantation	CI
Nematode	4.15	*(3.24, 5.83)*	5.45	*(3.97, 8.29)*	2.67	*(2.09, 3.29)*
Abbreviata	0.10	*(0.04, 0.21)*	0.10	*(0.00, 0.29)*	0.11	*(0.00, 0.22)*
Amplicaecum	0.95	*(0.55, 1.47)*	1.13	*(0.55, 1.94)*	0.74	*(0.30, 1.63)*
Chitwoodchabaudia	0.09	*(0.02, 0.26)*	0.10	*(0.00, 0.29)*	0.07	*(0.00, 0.19)*
Maxvachonia	1.78	*(1.21, 2.84)*	2.77	*(1.84, 4.61)*	0.63	*(0.22, 1.11)*
Neoxysomatium	0.05	*(0.00, 0.17)*	0.10	*(0.00, 0.29)*	0.00	*(0.00, 0.00)*
Omeia	0.16	*(0.04, 0.35)*	0.10	*(0.00, 0.29)*	0.22	*(0.04, 0.56)*
Oxysomatium	0.36	*(0.17, 0.67)*	0.32	*(0.07, 0.77)*	0.41	*(0.11, 0.93)*
Rhabdias	0.22	*(0.07, 0.57)*	0.00	*(0.00, 0.00)*	0.48	*(0.15, 1.04)*
Waltonella	0.45	*(0.00, 1.35)*	0.84	*(0.00, 2.52)*	0.00	*(0.00, 0.00)*

Figure 5.2: Frequency distributions of nematodes per host type. Refer to Figure 5.1 in this chapter for location of host. The mean, sample size (n) and distribution parameter corrected for sample size (k) added on the top right for each corresponding host. These variables were then used to generate negative binomial distribution curves, which were overlaid onto the frequency counts.



$F_{(16,20)}$= 0.13, p-value < 0.01), but the amount of variation differed between host family, as dicroglossids were more variable in forest, while microhylids showed more variation in plantations. Where dicroglossids did not show significant variation in foot length (F-test: $F_{(19,19)}$= 1.76, p-value= 0.225) or crus length (F-test: $F_{(16,20)}$ = 0.390, p-value= 0.06) between habitat types, microhylids did (foot: F-test: $F_{(16,20)}$ = 0.191, p-value < 0.01; crus: F-test: $F_{(19,19)}$= 4.21, p-value < 0.01), with the plantation species exhibiting a higher variation for both measurements. The morphological measurements were highly correlated with one another

Figure 5.3: Comparison of (a) snout-ventral length, (b) crus length, (c) thigh length and (d) foot length of host species found in oil palm plantation (*F. limnocharis* and *M. heymonsi*) and forest sites (*L. blythii* and *M. mantheyi*). Full scientific names can be found in host names of Figure 5.1. Hosts measurements were compared in pairs (*F. limnocharis* vs *L. blythii*; *M. mantheyi* and *M. heymonsi*). The resulting p-values and degrees of freedom are included in the figure above corresponding pairs. Thick lines represent median values with the top and bottom of the boxes representing upper and lower quantiles. Dotted lines illustrates the spread of the data (minimum and maximum values), while open circles are outliers in the data.



(Table 5.3), and based on principal component analysis, snout-vent length came out as most closely correlated with the first principal component (Table 5.4).

I found a significant host family, host sex and host size effect for overall parasite prevalence (Table 5.5). Microhylids had higher infection rates

Table 5.3: Correlation coefficients of morphological measurements of amphibian hosts collected from forest and plantation sites. Snout-ventral length = SV, thigh length = Thigh, crus length = Crus and foot length = Foot

Morphological measurement	SV	Thigh	Crus	Foot
SV	1.00	0.968	0.975	0.974
Thigh	0.968	1.00	0.977	0.974
Crus	0.975	0.977	1.00	0.975
Foot	0.974	0.974	0.975	1.00

Table 5.4: Loadings from principal component analysis of morphological measurements of amphibian hosts found in forest and plantation sites

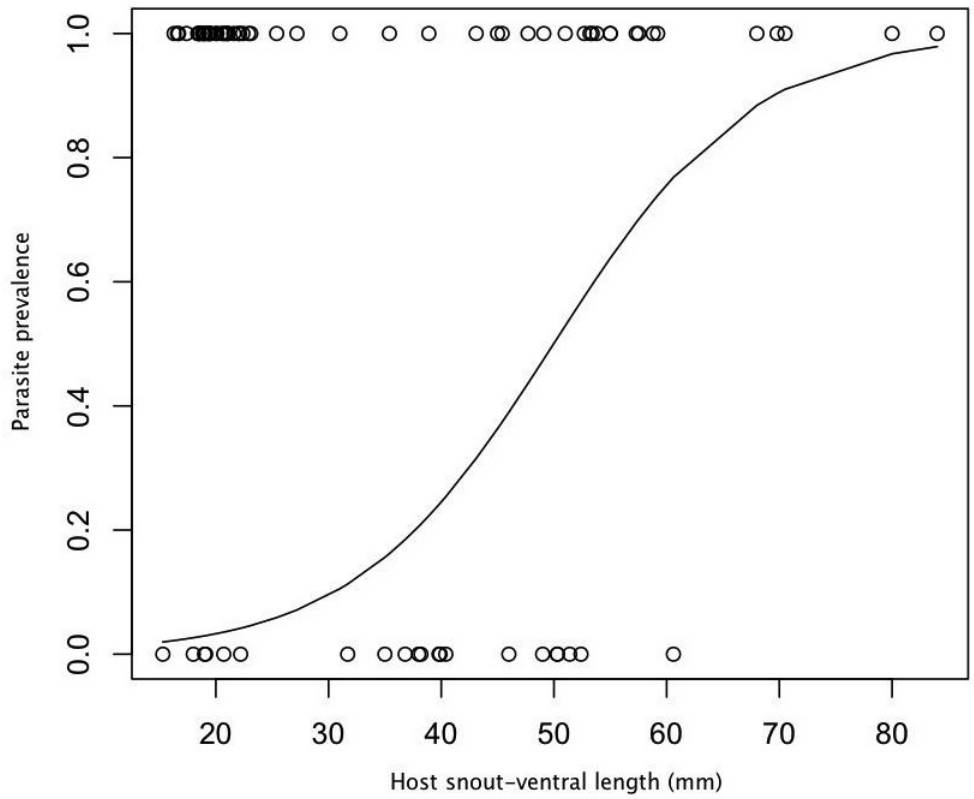
Morphological measurements	PC1	PC2	PC3	PC4
Snout-ventral length	-0.694	0.708	0.122	-0.058
Thigh length	-0.327	-0.295	-0.507	-0.741
Crus length	-0.371	-0.204	-0.615	0.665
Foot length	-0.523	-0.609	0.592	0.068

(42%) compared to dicroglossids (32%), while a higher proportion of male hosts were infected (48%) compared to females (25%). Based on the minimum adequate model, host body size had a positive relationship with parasite prevalence (estimated coefficient= 4.89 [SE 1.86]). The fitted curve of parasite prevalence against host body size exhibits a typical logit distribution (r-squared = 0.216), with prevalence increased rapidly up to snout-vent length of 65mm, at which point the rate of change decreases to a high prevalence value (Figure 5.4). The minimum adequate model for parasites with direct life-cycles indicated a significant interaction between habitat and host family (Table 5.5). A higher proportion of plantation microhylid species (*Microhyla heymonsi*) were infected (20%), followed by dicroglossid forest species (14%), dicroglossid plantation species (10%)

Table 5.5: Model selection for generalised linear model with binomial errors of parasite prevalence against habitat type (habitat), host sex (sex), host family (family), host snout-vent length (sv) and interaction terms for all the variables. Models were generated for overall parasites, parasites with direct and indirect life-cycles. Table shows the variables dropped from the maximal model, the deviance and degrees of freedoms of each model, including the Chi-square values and p-values from comparing models after dropping each variable without replacement. N (number of individuals sampled)=79

Variable dropped	**Model Deviance**	**Degree of Freedom**	**Chi-square**	p**-value**
Overall Prevalence				
none	70.2			
family:habitat	66.5	1	0.092	0.761
family:sex	66.6	1	0.092	0.762
family:sv	68.2	1	1.62	0.203
habitat:sv	69.0	1	0.808	0.369
habitat:sex	71.3	1	2.25	0.133
habitat	74.0	1	2.75	0.097
sex	79.7	1	5.67	0.017
sv	86.4	1	6.71	0.010
family	91.5	1	5.08	0.024
Direct life-cycle Prevalence				
none	95.3			
habitat:sv	95.3	1	0.017	0.896
family:sv	95.9	1	0.608	0.436
sv	95.9	1	0.042	0.838
family:sex	97.8	1	1.86	0.172
habitat:sex	101	1	3.35	0.067
sex	102	1	0.419	0.517
family:habitat	107	1	5.67	0.017
habitat	108	1	0.926	0.336
family	109	1	1.05	0.306
Indirect life-cycle Prevalence				
none	75.7			
habitat:sex	75.7	1	0.008	0.928
family:habitat	75.8	1	0.039	0.844
family:sv	75.9	1	0.115	0.734
habitat:sv	77.3	1	1.43	0.232
family:sex	78.7	1	1.43	0.232
sex	80.3	1	1.54	0.214
family	82.8	1	2.54	0.111
sv	85.1	1	2.29	0.131
habitat	104	1	18.7	$1.49e^{-05}$

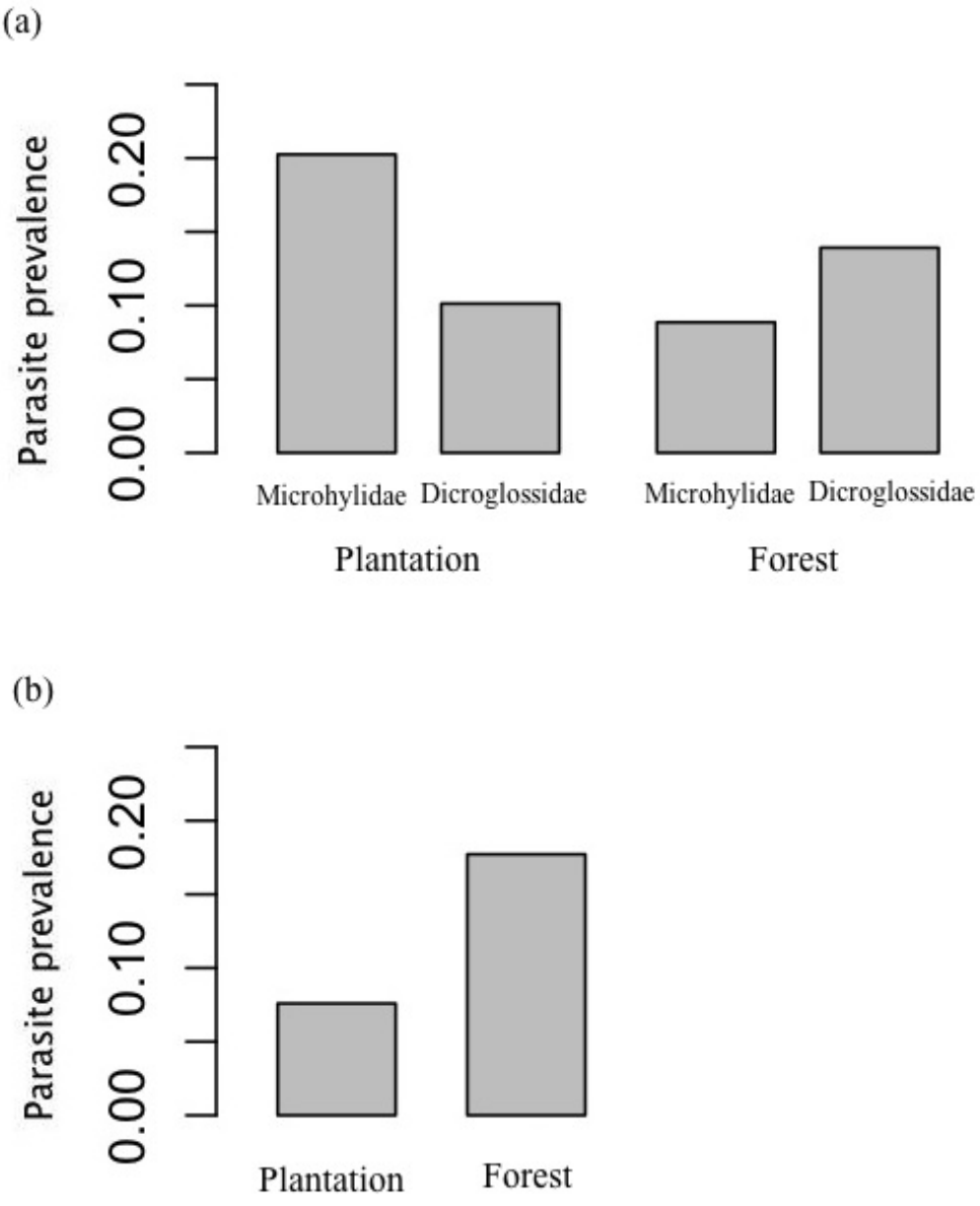
Figure 5.4: Graph showing fitted values (solid line) of the minimal model with binomial errors generated using a GLM applied on parasite prevalence. The x-axis is host snout-vent length (mm). Open circles are observed values.



and microhylid forest species, respectively (9%) (Figure 5.5a). A significant main effect of habitat was found for prevalence of parasites with indirect life-cycles (Table 5.5), with a significantly lower prevalence found in hosts from plantation sites (8%) compared to those found in forest sites (18%) (Figure 5.5b).

Based on the minimal adequate zero-truncated model, overall parasite intensity was significantly affected by habitat type and host snout-vent length (Table 5.6). Forest species had higher parasite intensities than those from plantation sites (Figure 5.6). Similar to overall parasite prevalence, overall parasite intensity also showed a positive relationship

Figure 5.5: Barplot showing the proportion of hosts infected by parasites with (a) direct life-cycles and (b) indirect life-cycles. The minimal model for prevalence of parasites with direct life-cycles showed a significant habitat and host family interaction. The minimal model for prevalence of parasites with indirect life-cycles showed a significant main effect of habitat (Refer to Table 5.5).



with host snout-vent length (estimated coefficient = 0.640 [SE 0.290]; r-squared=0.207; Figure 5.7). I found a significant interaction between host family and host snout-vent length against intensity of parasites with direct and indirect life-cycles (Table 5.6). While the intensity of parasites with direct life cycles was not significantly affected by habitat, models of parasites with indirect life cycles did reveal a significant habitat effect, with low intensities seen in hosts from plantation sites (Figure 5.8). The fitted model for intensity of parasites with direct life-cycles explained 64.7% of the overall variation, with a positive relationship of microhylid body size (estimated coefficients=0.27 [SE 0.04]) and a negative relationship with dicroglossid host body size (estimated coefficients=-3.91 [SE 0.49]) (Figure 5.9a). Indirect life-cycle parasite intensity fitted model explained 20.8% of the overall data variation, but exhibited the opposite pattern with direct life-cycle parasites; a negative relationship with microhylid body size (estimated coefficients=-0.19 [SE 0.11]) and a general positive relationship with dicroglossid body size (estimated coefficient=0.370 [SE 0.63]) after a sharp increase of intensity at snout-vent length of 50mm (Figure 5.9b).

Table 5.6: Model selection for zero-truncated GLMs of parasite intensity against habitat type (habitat), host sex (sex), host family (family), host snout-vent length (sv) and interaction of independent terms. Models generated for overall intensity, intensity for parasites with direct and indirect life-cycles. Table shows the variables dropped from the maximal model, AIC values, ΔAIC and log-likelihood. Model selection was done by dropping each term and comparing AIC values. ΔAIC is the difference between the AIC value of the previous model and the current model, with a value of ≥ 2 taken as a significantly worst fit to the data.

Variable dropped	**AIC**	**Δ AIC**	**Log-likelihood**
Overall intensity			
Maximum model	263		-121
Max .-family:sex	262	-1	-121
Max .-habitat:sex	261	-1	-122
Max .-family:sv	260	-1	-122
Max .-habitat:sv	263	2	-124
Max .-habitat:family	262	-1	-124
Max .-sex	260	-2	-124
Max .-family	260	0	-125
Max .-habitat:sv	260	-1	-126
Max .-sv	262	3	-128
Direct parasite intensity			
Maximum model	168		-74
Max .-family:sex	167	-1	-74
Max .-habitat:sex	165	-2	-75
Max .-sex	165	0	-76
Max .-habitat:sv	164	-1	-76
Max .-habitat:family	163	-2	-76

Continued on next page

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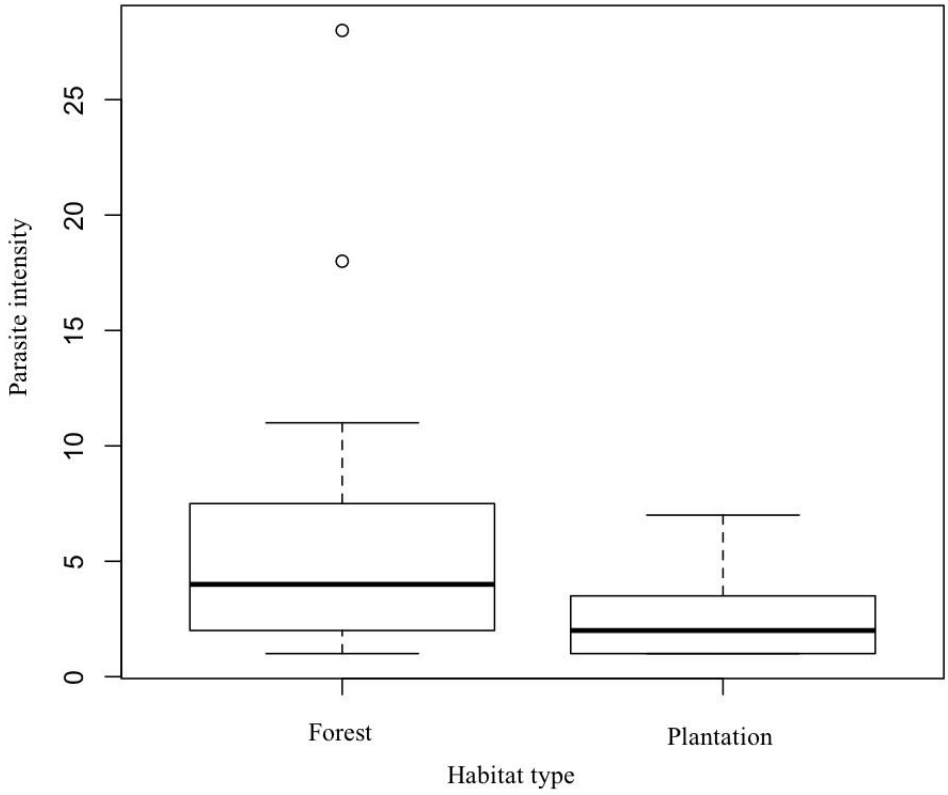
Variable dropped	AIC	Δ AIC	Log-likelihood
Max .-habitat	161	-2	-76
Max .-family:sv	198	37	-96
Indirect parasite intensity			
Maximum model	102		-41
Max .-habitat:family	101	-2	-41
Max .-habitat:sex	101	1	-43
Max .-habitat:sv	99.2	-2	-43
Max .-family:sex	97.7	-2	-43
Max .-sex	98.4	1	-44
Max .-habitat	103	5	-48

Nematodes from plantation hosts showed a 26% higher diversity index value compared to those infecting forest hosts. There was also a higher evenness in nematode infection in plantation sites compared to forest sites. However, the lowest diversity and evenness was found in *Microhyla mantheyi*, while the other host species exhibited similar index values. There was little difference in parasite diversity indices between male and female hosts (Table 5.7). Community composition differed significantly between forest and plantation hosts (ADONIS: $F_{(56,1)}= 2.35$, p-value=0.03). Parasite composition between host species were also significantly different (ADONIS: $F_{(54,3)}= 4.94$, p-value<0.001). The lung parasite, *Rhabdias* was only found in plantation host, specifically, *Fejervarya limnocharis*. *Neoxysomatium* and *Waltonella* nematodes were found only in hosts from forest sites, more specifically, *Limnonectes blythii*. *Chitwoodchabaudia* nematodes were found only in the Microhylid host species (Figure 5.10).

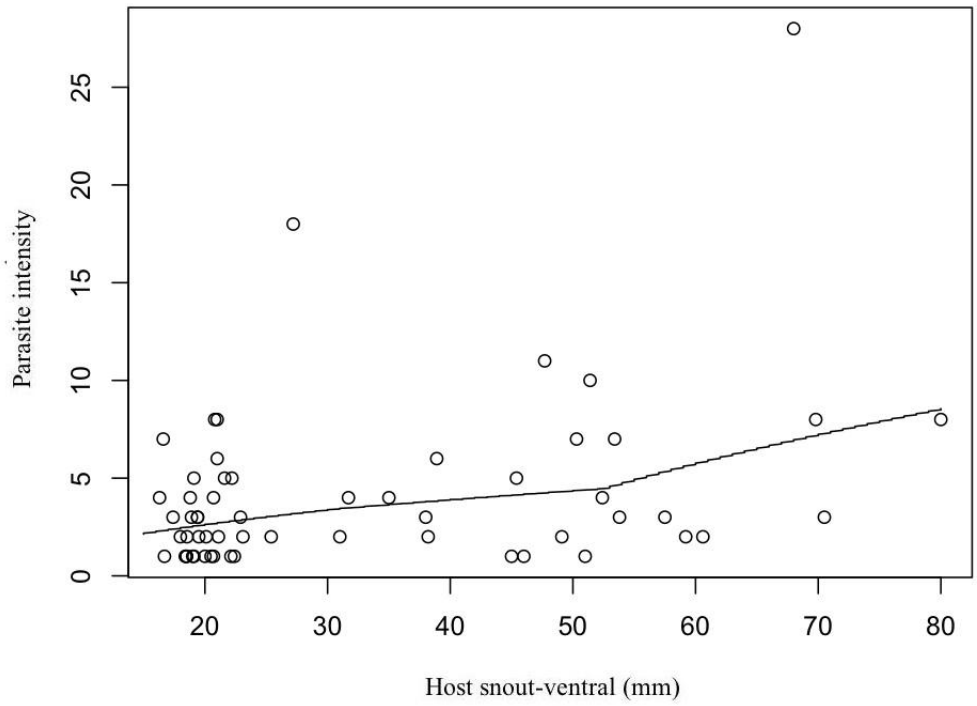
Figure 5.6: Boxplot of overall parasite intensity against habitat type (Forest and Plantation). The thick line represents the median value, while the top and bottom of the boxes represent the upper and lower quantiles, respectively. The whiskers represent the spread of the data (minimum and maximum values), with the open circles representing outliers in the data.



5.4 Discussion

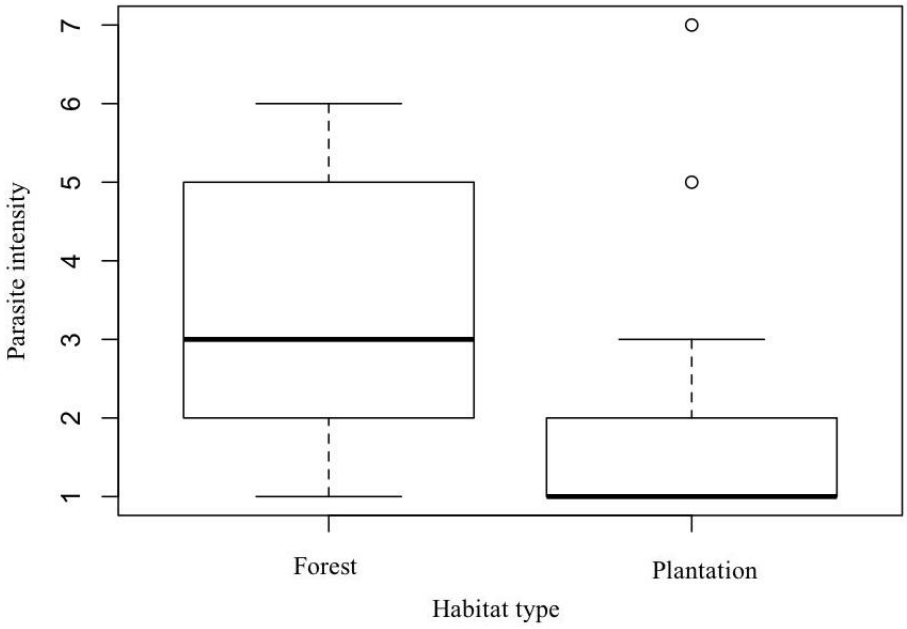
I did not detect *Batrachochytrium dendrobatidis* infection from either of the oil palm plantations or forest amphibians sampled. This implies that *Bd* is either absent from the area or only present at a low prevalence. A previous survey of amphibians across Peninsula Malaysia found ten out of a sample of 127 individuals positively infected with *Bd* (Savage *et al.*, 2011). Some of the areas Savage et al surveyed were close to my current

Figure 5.7: Graph showing fitted values (solid line) of the minimal model with binomial errors generated using a GLM applied on overall parasite intensity. The x-axis is host snout-vent length (mm). Open circles are observed values.



study sites and they found a positive infection from only one individual in that area. On the basis of the results from this study and the data from Savage et al in 2011 we can be reasonably confident that *Bd* was not highly prevalent within my study system. In contrast to *Bd*, the other generalist microparasite, *B. ranarum*, along with endoparasitic nematodes, were found to infect amphibians across both habitat types. A number of nematodes within my sample were found with developing eggs, but some were not, indicating that the amphibian species used for this study functioned as both intermediate and definitive hosts. Not all nematode genera exhibited a generalist approach when it comes to host species. For example, *Rhabdias* was only found in one of the plantation amphibian species and is

Figure 5.8: Boxplot of intensity of parasites with indirect life-cycles against habitat type (Forest and Plantation). The thick line represents the median value, while the top and bottom of the boxes represent the upper and lower quantiles, respectively. The whiskers represent the spread of the data (minimum and maximum values), with the open circles representing outliers in the data.



the only nematode genus within the sample known to cause severe pathology (Koprivnikar *et al.*, 2012). The significant differences in parasite genus composition fit with the predictions laid out in the introduction, as well as emphasizing the need for studying the effects parasites present in altered habitats have on their host population.

The use of straightforward comparison analysis failed to detect subtle host effects on parasites. When additional information was added, parasite prevalence showed an association with several characteristics of the host (host family, body size and sex). Overall intensity was also influenced by the host, in terms of size. The results from my models suggests that the important factor for a parasite is its immediate environment, i.e. its host, rather than the overall habitat. There are a multitude of reasons

Figure 5.9: Graph showing fitted values (solid and dashed lines) of the minimal adequate zero-truncated model with poisson errors of intensity of parasites with (a) direct and (b) indirect life-cycles. Relationships between parasite intensity and host snout-vent length (mm) differed between host family. Circles represented observed values. The lines and circles corresponding to each family are found at the top right corner of each graph.

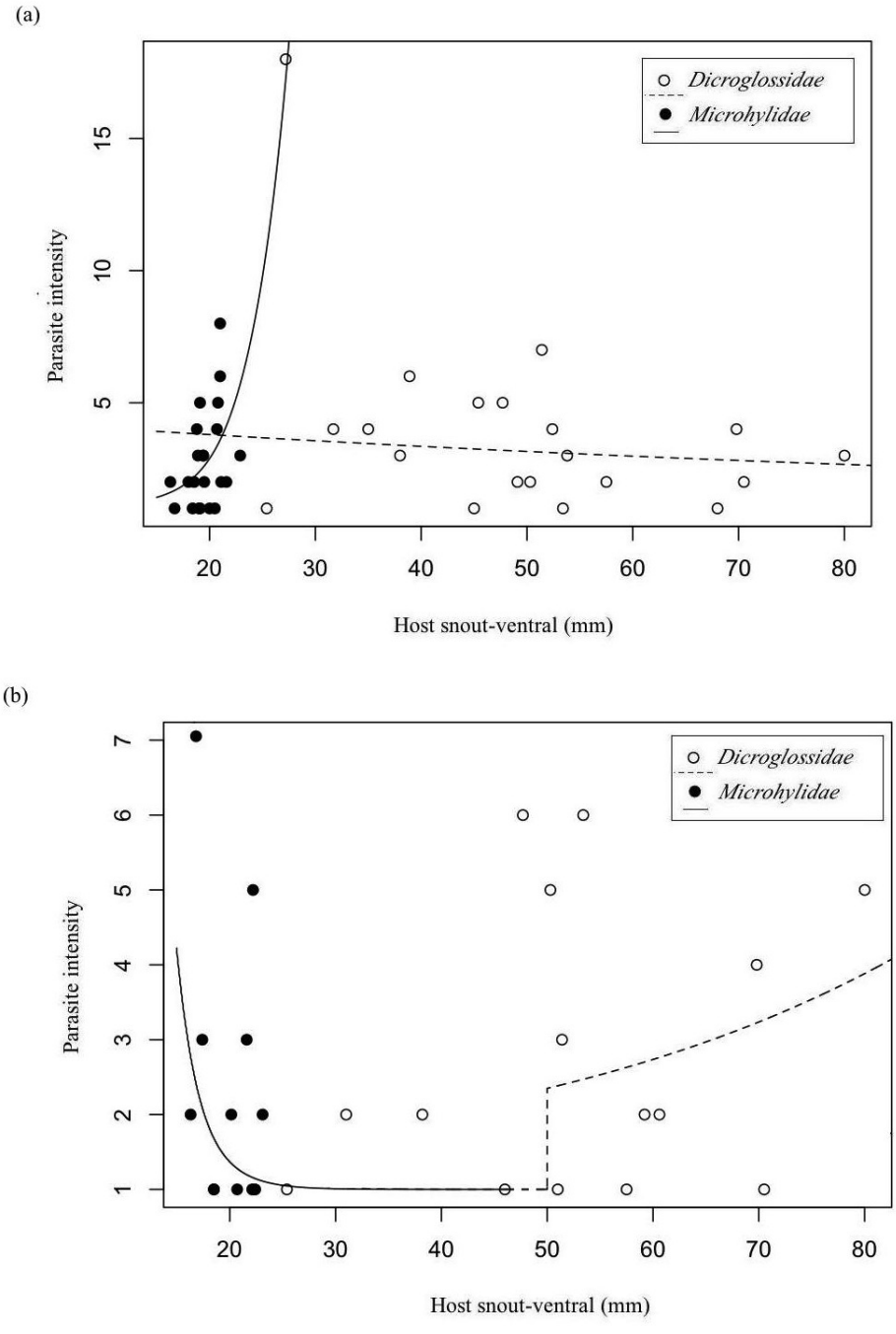
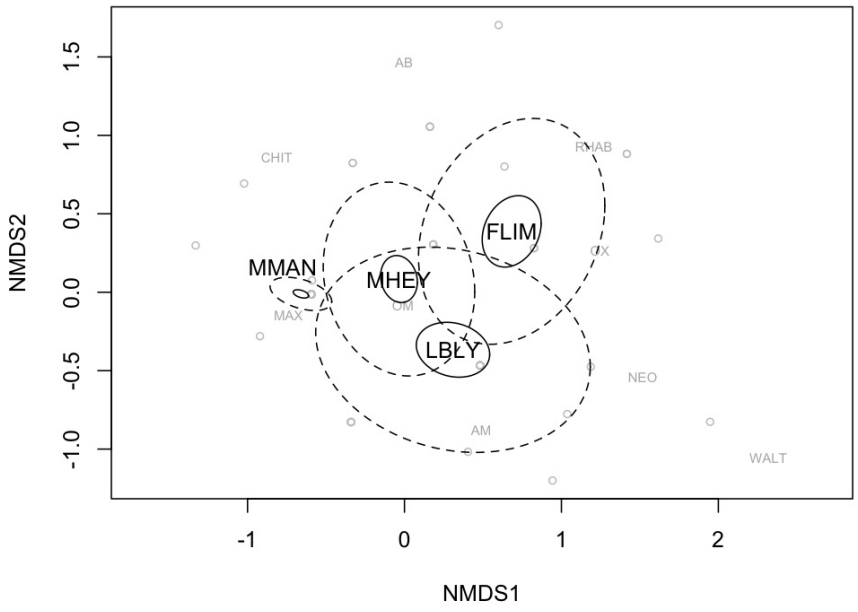


Table 5.7: Total number of nematodes found, Maximum Brillouin Index, Brillouin Index and evenness of nematodes found in forest (*Limnonectes blythii* and *Microhyla mantheyi*) and plantation (*Fejervarya limnocharis* and *Microhyla heymonsi*) hosts

Host	**Nematode count**	**Brillouin Index**	**Max Brillouin Index**	**Evenness**
Limnonectes blythii	99	1.40	2.04	0.69
Fejervarya limnocharis	30	1.19	1.81	0.66
Microhyla mantheyi	70	0.31	1.99	0.16
Microhyla heymonsi	42	1.24	1.89	0.66
Male host	169	1.50	2.08	0.64
Female host	148	1.40	2.03	0.72
Overall forest	92	1.33	2.09	0.69
Overall plantation	69	1.60	1.99	0.80

why host type may play an important part in the susceptibility to parasite infection. For example, host body size followed a positive relationship with parasite infection simply because of increased space for more parasites or increased transmission due to foraging and feeding, a pattern also found in other host-parasite systems (Poulin, 2000; Kelehear *et al.*, 2012). Similarly, host sex and/or species can also greatly influence a hosts immunity and therefore affect both the probability of infection and its intensity (Folstad & Karter, 1992; Poulin, 1996; Atkinson *et al.*, 2000). When I separated intensity of parasites based on life histories, both directly and indirectly transmitted parasites showed an interaction between host family and host body size, indicating that the relationship between intensity and body size is dependent on host type, further emphasizing the importance of host characteristics to all types of parasites. It must also be noted that the pattern exhibited between directly and indirectly transmitted parasites differed, for example, microhylid body size was positively associated with intensities of directly transmitted parasites, but negatively associated

Figure 5.10: Non-metric Multidimensional Scaling (NMDS) of parasitic nematode assemblages in amphibian host species from forest (MMAN and LBLY) and plantation (MHEY and FLIM) sites from the 2010 collection (final stress value=0.107, number of dimensions=2). Open circles represents amphibian host and distance on the ordination reflects dissimilarities in nematode assemblages based on Binomial coefficients. Abbreviations in grey represent nematode species, abbreviations in black represent amphibian host species (refer to Appendix A.1 for full host species names and Appendix D for full parasite genus names). Solid and dotted lines represent the standard errors and standard deviation for each host respectively.



with intensities of indirectly transmitted parasites. As directly transmitted parasites generally exhibit density dependent transmission, parasite intensity should increase with host densities, while indirectly transmitted parasites typically exhibiting frequency dependent transmission would be affected by the frequency of infected individuals. Therefore, the differences in relationships between intensity and host characteristics can differ drastically based on the life history of the parasite. It is then important that both host and parasite characteristics are taken into account when studying the effects of habitat loss on host-parasite relationships.

A number of authors have documented detrimental effects of agricultural conversion on amphibian body condition (Karraker & Welsh Jr, 2006; Burgett *et al.*, 2007; Brodeur *et al.*, 2011). Such patterns were not evident in this study as body size variance for plantation amphibians was not consistently less than forest hosts and measurements of body size for plantation hosts were within the range of the norm for both species (Belabut, 2012, N. Ahmad, 2012, *pers. comm*). Additionally, directly transmitted parasite prevalence and intensity models did not show a significant habitat effect, as predicted in the Introduction of this chapter. If we assume that parasites with direct life-cycles in this study were driven by density dependent transmission patterns, the comparable overall host abundance values found in Chapter 2 between plantation and forest sites would explain the lack of difference in infection from these types of parasites. The model of intensity for directly transmitted parasites did show a significant habitat and host family interaction, indicating that parasite intensity was dependent on what the host is as well as within which habitat. Although the importance of host can be inferred from the model, as comparisons between habitat types were made from the same family, the same inference cannot be, confidently, made with habitat effects. This is because I was not able to compare the same species between different habitats due to host communities between the two habitats being so distinct. Therefore, the effect of host species and habitat could not be teased apart and any inferences made based on a significant habitat term needs to be taken with caution. For example, the overall intensity model and those of parasites with indirect life cycles. If habitat was an important factor, the low overall intensity in plantation sites would imply that the agricultural practices in oil palm plantations may not have a detrimental effect on plantation host immunity. As with body condition, this would contradict past studies made in other agricultural habitats (Christin *et al.*, 2003; Rohr *et al.*, 2008), and would indicate that amphibians found in this habitat type are able to cope with the current activities and management of oil palm cultivation.

However, when both intensity and prevalence were separated based on

parasite life-cycles, plantation sites exhibited low prevalences and intensities of indirectly transmitted parasites. Although this may benefit the individual amphibian, it might indicate a wider problem with agricultural conversion. Indirectly transmitted parasites generally need one or two intermediate hosts to complete their life cycle, typically including invertebrates. An impoverished invertebrate fauna, which has been reported previously in plantations (Turner & Foster, 2008), would lead to a reduced transmission of parasites with complex life-cycles to the amphibian host. Additionally, it also implies a reduced amount of the definitive host, usually birds, larger mammals or reptiles, within plantations. Parasites with complex life-cycles usually produce eggs within their definitive hosts, which are then passed to the first intermediate host via their faeces. The parasite then infects as many intermediate hosts it needs to complete its life-cycle, after which, the definitive host is again infected, typically by ingesting the last intermediate host. One caveat to the suggestion that this pattern is linked to transmission rates is the comparable prevalence values of *B. ranarum* between forest and plantation sites. Although *B. ranarum* is thought to be transmitted via ingestion of infected food, it has been cultured previously from leaf litter and soil samples (Hutchison & Nickerson, 1970). Therefore, it is possible that this pathogen might also be directly transmitted via contaminated soil, and would then follow a similar pattern as directly transmitted nematodes.

The impoverished definitive host fauna in plantation sites could also explain the difference in parasite frequency distribution between the two habitat types. The most common distribution found in both free-living and parasite populations is an aggregated one (as seen in both plantation host species). In terms of parasite infection, this would mean a large number of hosts with low parasite loads, while a small number of hosts with very high parasite loads (Bush *et al.*, 2001). In contrast, the random distribution patterns seen in both forest host species indicated that the probability of infection for individuals in those species was relatively equal, a pattern not commonly found in natural populations (Bush *et al.*, 2001). The most favoured explanation for the existence of this type of distribution pattern is from Williams (1987), which states that uniform infections

are possibly due to regulation of parasites by the hosts immune system through intraspecific competition. I am unable to say if this is the case for my system, as along with not measuring immunity within hosts, a lowered immunity in plantation hosts compared to forests hosts would contradict the lowered intensity in plantation host species. Alternatively, hosts with high macroparasite burdens can be ineffective in avoiding predators, evident in studies of other animal taxa (Moore, 2002; Hatcher *et al.*, 2006), which could lead to a lowered probability of sampling amphibians with high burdens in forests sites, where predators are diverse and in higher numbers compared to altered habitats (Peh *et al.*, 2005; Azlan J, 2006; Wanger *et al.*, 2009).

As previously discussed, host characteristics are important factors affecting parasite prevalence and intensity, and due to the habitat-host species conundrum, significant habitat terms may actually be a significant effect of host species. The problem then lies in transferring the patterns seen from subsequent analyses across the general habitat. The same can be said for parasite diversity measures, as a low diversity value from a single species (*Microhyla mantheyi*) resulted in forest sites having a lower parasite diversity compared to plantation sites. Due to the results from this single species, a low parasite diversity value in forests may not be a general pattern, as diversity is linked to a particular species. If we then collect different host species, diversity values may, then, differ. Nevertheless, converting natural habitats into oil palm has the potential to change the amphibian community composition substantially (Chapter 2), and if the patterns of habitat effects are truly an effect of species, then the newly altered habitat will change in such a way as to reduce indirectly transmitted parasite prevalence and intensity, but maintaining diversity and infection of directly transmitted parasites.

Although this study is in no way exhaustive of the possible effects habitat loss would have on host-parasite relationships, it does reveal some interesting results and identifies possibly ways in effectively measuring amphibian host-parasite relationships in agricultural habitats. For example, host body condition and possibly immunity may not be ideal habitat effect measurements, as amphibians dominating fully established altered

habitats are adapted to thrive in such environments. Similarly, parasite diversity was shown to be affected by host species and therefore, the results from this type of index cannot be transferred to the general habitat. As with the study conducted on the host species (Chapter 2), this study also indicated the danger of using direct comparison methods to measure parasite prevalence and intensity across different habitats. Direct comparisons failed to take into account the important effects of the host and associated characteristics. Additionally, the models from different parasite types is evidence of how host-parasite patterns can also differ based on the parasites life history. Hence, future studies will not only need to take into account host characteristics, but also the characteristics of the parasite in question. This study shows how studying parasites may unveil a wider problem altering habitats would have on ecosystems. Although the study is limited based on the inability to compare similar species between habitat types, the results support surveys done on other animal taxa in the same system. The presence of parasites with complex life-cycles can possibly indicate a highly diverse habitat, as well as suggest that different host groups are not isolated. Using non target species to obtain data of target species is a viable possibility for wildlife monitoring. For example, leeches have been discussed as a mammal monitoring tool (Schnell *et al.*, 2012) and the non-selective feeding of amphibians (Meharg *et al.*, 1990) could be a possible way for invertebrate surveys through stomach flushing. The advantage of studying parasites with monitoring surveys in mind, is the ability to monitor the presence and condition of multiple host species within a particular ecosystem.

Chapter 6

Overall discussion

6.1 Discussion

6.1.1 Main findings

This project was undertaken to evaluate the impact of oil palm plantations on amphibians in Peninsula Malaysia, with a general idea for improving plantation management for overall amphibian diversity in mind. This dissertation has compared different biodiversity measurements between habitat types, macro- and microhabitats associated with different amphibian species, identified the breeding habits of amphibians in plantations, along with evaluating parasite infections of hosts found in both habitat types. This study has showed how different biodiversity measurements (species richness, abundance and community composition) can give contrasting results. Oil palm plantations had comparable species richness and overall abundance counts to forested areas, while amphibian communities were consistently distinct across macrohabitats surveyed (stream, riparian, terrestrial). The types of amphibians found in plantation sites were species readily found in other disturbed habitats. Although abundant species exhibit spatial partitioning between each other, temporary pool breeding species in plantation were not specific to that particular habitat. In addition, body condition and overall parasite prevalence did not show any evidence of amphibians in plantations being adversely affected by agricultural practices, although the lowered amount of infection of indirectly transmitted parasites indicated a wider problem within plantation sites.

6.1.2 Limitations

A number of caveats need to be noted regarding the collection of studies within this thesis. Firstly, in the overall survey, I did not detect any species from the Rhacophoridae genus in my forest sites., even though species from this genus have been found in the same area previously (Onn *et al.*, 2010) This highlights a universal problem in biodiversity surveys (Sewell *et al.*, 2010; Schmidt, 2003). Although I used species richness estimates that take into account undetected species, such analyses do not identify the species that were undetected, meaning that the community composition dataset is by no means an exhaustive list of what could be present within the habitats surveyed. Studies of a similar nature also showed much higher estimates for species richness compared to the corresponding species list, either through accumulation curves or statistical analyses (Struebig *et al.*, 2008; Gillespie *et al.*, 2012). However, with such little overlap between forest and plantation sites even after intensive-repeated surveys, this limitation should not detract from the overall conclusion that the two habitats had distinct amphibian communities.

Another source of weakness seen in the third chapter is the small sample size of forest pools. With a small sample size, any generalisations about the overall structure of forest temporary pools based on this study need to be treated with caution. Models of amphibian occupancy from forest sites with varied detection probabilities did not converge, possibly due to sample size, which affects the confidence of my detection probability estimates, and therefore, occupancy estimates as well. The current study was also unable to analyse the difference in breeding site selection between forest and plantation amphibian species. If species from the two communities showed preferences for a similar aspect of a pool, then I would be able to argue that temporary pool breeders exhibit generalist behaviours when it comes to breeding site choices. In addition, it would also be possible to discuss the possibility of plantations providing potential breeding sites for forest species. Alternatively, if forest species did show specificity to forest pools, plantations may not be suitable for amphibian breeding and discuss the plausibility of adding those chosen characteristics in plantation

management schemes.

A low number of individuals in Chapter 4 limited my ability to adequately describe microhabitat associations of all species within forest and plantation communities. Therefore, only a subset of species were studied, which can be a problem when trying to generalise the associated patterns across the whole habitat. For those that were studied, the small sample size can affect the strength of the association with each microhabitat variable for that particular species and extreme values of one or two individuals can affect the outcome of the ordination.

Finally, in the last data chapter, a number of important limitations need to be considered. The current study has only examined nematodes at the genus level. This affects the results, as some parasites may exhibit host specificity and therefore, the patterns of species diversity and community composition between habitat types might be different if nematodes were to be identified to the species level. Another caveat in this chapter is the confounding effects of host species and habitat type. The inability to tease out these two effects led to difficulty in generalising the patterns resulting from associated analyses across the overall habitat. These include the low parasite intensity and low indirect parasite infection measures. However, if the lowered intensity of parasites overall in plantation sites is associated with habitat, this would be consistent with the lack of apparent amphibian body deterioration in these putatively less suitable habitats. In addition, the lowered indirect parasite prevalence and intensity are in agreement with surveys previously carried out on invertebrate and predator abundance in altered habitats, which can also explain the difference in parasite frequency distributions.

6.1.3 What has been achieved in this thesis?

The findings from this thesis have a number of important implications for future monitoring schemes. One of which is the need to explicitly determine the definition of "conservation value". The surprising results from Chapter 2 imply that oil palm cultivation does not adversely affect amphibian communities in Peninsular Malaysia when these are assessed on

the basis of species richness and abundance values. This contrasts with past studies of biodiversity in the same system (Koh & Wilcove, 2008). The diverse feeding strategies of tadpoles and specific microhabitat associations of adults also suggest that plantations can support communities without strong competition for resources. Additionally, amphibians in plantations appeared to be reasonably healthy: there was no deterioration of body condition, or any increase in overall parasite infection. Based on these results, we could conclude that amphibians are common, relatively diverse and healthy in plantation sites. However, if we define conservation value as the presence of endemic species associated with forests, constant heterogeneity between all macrohabitat types (streams versus terrestrial) and a rich abundance of different hosts within the same habitat, then plantation sites fail to come up to such standards. There is, therefore, a definite need for future studies to make clear definitions on what needs to be conserved. On a personal note, I believe that maintaining natural ecosystem functions, for example, by preserving functional diversity rather than species richness, should be the main measure of conservation value.

Another important practical implication is identifying the proper methodology at the right level. Whilst this study is by no means an extensive one, the intensive nature of the surveys allow amphibian communities to be compared at a variety of scales, and the conclusions reached vary depending on the scale. For example, at the wider habitat level, abundance values were similar between the two habitat types. However, the same cannot be said at the macrohabitat level, where plantation streams had considerably fewer amphibians than forest streams. Amphibian species richness was also comparable at all levels. The surprisingly high diversity in plantation sites may lead to difficulty in identifying significant habitat effects at the microhabitat level, as amphibians in plantations exhibited similar ecomorphologies with forest species, which in turn may lead to similar microhabitat associations. Therefore, comparing microhabitats between plantation and forest species may not be advisable for future monitoring studies. Community composition, however, did show significant and important differences at the overall habitat and macrohabitat

levels. At the wider habitat level, plantations provided structurally adequate pools for amphibians, but amphibians in this area were generalist breeders and it appears that specialist breeders found in forests may not be able to utilize the same pools. A more comprehensive result was also found when nematodes were separated based on life histories. In addition, using statistical models that took into account additional terms that may affect parasite infection ensures that we are interpreting the data correctly. In conclusion, using overall comparison analyses can be misleading, as these types of comparisons can fail to detect the subtle differences that can make a major difference in the way plantations are monitored and managed in the future. Future monitoring schemes should take into account the scale at which research should be conducted and utilize statistical tools that would enable researchers to answer a particular question correctly.

As the expansion of altered habitats throughout Southeast Asia progresses, research on the amphibians living in these habitats should also be ongoing. This study adds to the understanding of the breeding habits and host-parasite relationships of amphibians in altered habitats. It was surprising to note that amphibians in plantations did not show any obvious adverse effects from plantation cultivating practices, as abundances, body condition and comparable parasite infections were found. This contradicts other studies of agricultural practices and amphibian populations (Karraker & Welsh Jr, 2006; Burgett *et al.*, 2007; Brodeur *et al.*, 2011). Although no severe pathology from parasite infection was found, the genus *Rhabdias* was found in plantation sites. Although at low intensities, *Rhabdias* may not be detrimental to the overall health of an individual, heavy infections can be detrimental and increases mortality. Amphibians in plantation sites also showed adaptations to living in exposed habitats by selecting deep pools. This result does not make them specific to oil palm, but it does show a degree of selectivity by species previously thought to be opportunistic breeders that would breed in any water body. In addition, amphibians in plantations were also found to utilize the microhabitats available to them and show a degree of partitioning in terms of tadpole feeding guilds. This suggests that plantations are able to support a relatively diverse amphibian community, but it should be noted, a

substantially distinct one from forested habitats.

Despite the resourceful nature of amphibians in plantation sites, the impoverished stream amphibian numbers and their associations with open areas emphasizes the importance of future management of streams at these sites. In addition to this, the low prevalence of indirect parasites suggests a wider issue of an impoverished invertebrate and predatory fauna. These two results were supported by past studies, where plantation are poor habitats for benthic stream macroinvertebrates (Azrina *et al.*, 2006), overall invertebrate abundance (Turner & Foster, 2008; Brühl & Eltz, 2010), and amphibian predators (Koh & Wilcove, 2008). The findings from this study provide some evidence that, although amphibians in plantations may not show obvious adverse effects from conversion, they can act as indicators for specific areas that require urgent management.

6.1.4 Future work

Future research in this field might include monitoring the effectiveness of different schemes put forward to oil palm plantation managers by a number of NGOs and conservation programmes since the start of this study. Bakewell et al (2012) published a number of case studies and subsequent how-to guides on managing plantations for increased biodiversity. One of the case studies is the *River of Life* project in Negeri Sembilan. The project was disbanded in 2008 but continued by certain plantations owned by Sime Darby. The project, however, is slow moving and the main objectives were to improve water quality, reduce erosion into streams and provide an opportunity to improve the companys image to the wider public. Although water quality is a key aspect to the ecosystem, it would be interesting to study the effectiveness of replanted riparian sites on amphibian populations. Additionally, plantations close to forest remnants may be able to connect their waterways through this riparian buffer and identify the possibility of incorporating forest species within plantation sites. Such a project may shed some light as to which forest species can be sustained in newly managed plantations and if there is any difference with plantations constructed after the riparian buffer legislation was put

in place.

A point made by Bakewell et al (2012) is that there is a lack of studies aimed toward identifying functional diversity in plantations. In Sabah, Borneo, a study on the functional diversity of amphibians in oil palm plantations is already ongoing in the S. A. F. E. experimental sites in Sabah, Borneo (http://www.safeproject.net/projects/biodiversity/species-interactions/functional-diversity-of-amphibians/). Here, the team is identifying different feeding habits, morphological characteristics and habitat preferences of amphibians across different habitat types, one of which, includes oil palm plantations. However, based on the comparisons made between this study and the one carried out by Gillespie et al (2012), generalizing the results obtained in Bornean sites across to those in Peninsular Malaysia can be problematic.

Although the effects of chemical compounds used in agricultural sites on amphibians have been extensively studied, it would be interesting to identify the effects of agrochemicals on the free-living stages of nematode parasites infecting amphibians. Such a study could explain the patterns of parasite intensity found in Chapter 5 of this thesis. These types of studies can also identify if a reduced parasite diversity can effect the overall ecological functioning of agro-ecosystems.

Future studies can also include a genetic aspect and there is a whole host of research questions that can be asked in this particular topic theme. Based on this study, I identified that plantation amphibians select for deep pools. As deep pools have the probability of filling up during every rain event, there is the question of using genetic analysis to identify if amphibians in these altered habitats exhibit breeding site fidelity. Genetic analysis can also evaluate the degree of isolation and genetic heterogeneity of amphibian populations in plantation habitats.

It is my hope that this research will serve as a base for future studies. It is important to note that although conserving highly diverse and unique habitats should always be the main objective, the welfare of human populations in developing countries is also an important issue. There are some groundbreaking studies out there to further our understanding of the natural world and the influence our actions and practices have on

it. The search for knowledge is ongoing and where mistakes were made in a practical sense, it is our responsibility to amend them for a sustainable future. We must now identify ways to better our understanding of how to monitor and manage areas that are being used to sustain an ever growing human population. After such time, we must then put the same knowledge into practice.

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Appendix A

Table A.1: Number of individuals found for each species per habitat sampled. Species abbreviations correspond to Fig. 4a-c. Threat status for each species are based on the IUCN red list criteria (http://www.iucnredlist.org/); DD-Data Deficient, LC-Least Concern, NT- Near Threatened

Species	Abbreviation	Plantation			Forest			Threat status
		Stream	Riparian	Terrestrial	Stream	Riparian	Terrestrial	
Bufonidae								
Duttaphrynus melanostictus	DMEL	1	9	7	-	-	-	LC
Ingerophrynus parvus	IPAR	-	3	-	-	3	8	LC
Phrynoides aspera	PASP	1	-	2	28	9	1	LC
Dicroglossidae								
Fejervarya cancrivora	FCAN	2	26	4	2	-	-	LC
Fejervarya limnonectes	FLIM	12	94	27	-	-	-	LC
Fejervarya nicobariensis	FNIC	25	4	11	-	-	1	LC
Limnonectes blythii	LBLY	-	-	-	28	13	-	NT
Limnonectes plicatellus	LPLI	-	-	-	-	1	1	LC
Limnonectes laticeps	LLAT	-	-	1	-	-	-	LC
Limnonectes malesianus	LMAL	-	-	-	11	6	-	NT
Occidozyga laevis	OLAE	-	-	-	3	1	-	LC
Microhylidae								
Kalophrynus pleurostigma	KPLEU	-	-	1	-	-	-	LC
Kaloula pulchra	KPUL	1	25	23	-	-	-	LC
Microhyla butlerii	MBUT	-	16	8	-	-	10	LC
Microhyla heymonsi	MHEY	-	28	63	-	3	2	LC
Microhyla inornata	MINO	-	-	-	-	2	1	LC
Microhyla mantheyi	MMAN	-	-	-	-	2	7	DD
Microhyla superciliaris	MSUP	-	-	-	-	-	4	DD
Ranidae								
Hylarana baramica	HBAR	-	-	-	-	-	5	LC
Hylarana erythrea	HERY	47	9	1	1	-	-	LC
Hylarana glandulosa	HGLAN	-	-	1	16	6	6	LC
Hylarana labialis	HLAB	-	-	-	73	20	12	DD
Hylarana miopus	HMIO	-	-	4	-	-	-	LC
Hylarana picturata	HPIC	-	1	-	8	5	-	LC
Oddorana hosii	OHOS	-	-	-	12	2	-	LC
Rhacophoridae								
Polypedates leucomystax	PLEU	2	4	49	-	-	-	LC
Total		91	219	202	182	73	58	

Appendix B

Figure B.1: Stream found in forest [(a) Kuala Lompat, (b) Bukit Rengit], along with a typical plantation stream, (c)

(a)

(b)

(c)

Appendix C

Table C.1: Quadratic linear models for pool water depth against visit number (1^{st}, 2^{nd}, 3^{rd} and 4^{th}) for pools used for breeding and non-breeding by amphibians

	Breeding present	**Breeding absent**
Coefficients		
Intercept (*SE*)	-3.60 (*6.27*)	-7.55 (*5.83*)
Visit (*SE*)	10.3 (*5.72*)	13.7 (*5.32*)
Visit^2 (*SE*)	-2.13 (*1.13*)	-2.74 (*1.05*)
Adjusted r^2	0.361	0.617
F-statistics	1.85	3.41
***p*-value**	0.462	0.357
Residual standard error	2.25	2.10
degree of freedom	1	1

Appendix D

Table D.1: Water depth of each pool in plantation sites (Lanchang and Sungai Mai) and forest sites (Kuala Lompat and Bukit Rengit) measured at each visit

Pool no.	**Site**	**Depth (cm)**			
		Visit 1	Visit 2	Visit 3	Visit 4
1	Lanchang	13	15	17	3
2	Lanchang	11	14	18	3
3	Lanchang	8	14	16	0
4	Lanchang	16	8	0	0
5	Lanchang	10	2	0	0
6	Lanchang	10	2	5	2
7	Lanchang	8	5	13	0
8	Lanchang	10	0	0	0
9	Lanchang	0	10	5	0
10	Lanchang	0	10	4	0
11	Lanchang	0	1	13	0
12	Lanchang	0	15	5	0
13	Lanchang	0	30	0	0
14	Lanchang	0	9	10	0
15	Lanchang	0	24	0	0
16	Lanchang	0	15	9	2
17	Lanchang	0	30	0	0
18	Lanchang	0	28	0	0
1	Sungai Mai	48	10	60	65

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Pool no.	**Site**	**Depth (cm)**			
		Visit 1	Visit 2	Visit 3	Visit 4
2	Sungai Mai	15	10	16	3
3	Sungai Mai	1	11	10	3
4	Sungai Mai	2	12	5	4
5	Sungai Mai	1	3	3	0.5
6	Sungai Mai	1	4	2	0
7	Sungai Mai	9	41	35	29
8	Sungai Mai	0	1	0	0
9	Sungai Mai	0	1	0	0
10	Sungai Mai	0	3	0	0
11	Sungai Mai	0	1	0	0
12	Sungai Mai	0	2	0	0
13	Sungai Mai	0	1	0	0
14	Sungai Mai	0	3	4	0.5
15	Sungai Mai	0	5	5	0
16	Sungai Mai	0	5	25	0
17	Sungai Mai	0	5	0	10
18	Sungai Mai	0	5	0	20
19	Sungai Mai	0	2	0	0
20	Sungai Mai	0	18	0	0
21	Sungai Mai	0	1	4	0
22	Sungai Mai	0	1	0	0
23	Sungai Mai	0	4	0	10
24	Sungai Mai	0	50	32	27
25	Sungai Mai	0	20	2	0
26	Sungai Mai	0	4	0	0
27	Sungai Mai	0	3	0	0
1	Kuala Lompat	10	50	58	38
1	Kuala Lompat	15	50	58	49
1	Kuala Lompat	3	30	49	49

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Pool no.	**Site**	**Depth (cm)**			
		Visit 1	Visit 2	Visit 3	Visit 4
1	Kuala Lompat	10	30	49	30
1	Kuala Lompat	50	90	60	49
1	Bukit Rengit	35	40	20	20
1	Bukit Rengit	13	20	15	20
1	Bukit Rengit	1	5	0	10
1	Bukit Rengit	0	60	30	0

Appendix E

Figure E.1: Temporary pools typically found in (a) forest and (b) plantation sites

(a)

(b)

Appendix F

Table F.1: Count of individuals detected during microhabitat surveys per species

Species	**Plantation**			**Forest**		
	Stream	Riparian	Terrestrial	Stream	Riparian	Terrestrial
Duttaphrynus melanostictus	1	6	3	-	-	-
Fejervarya cancrivora	2	1	1	1	-	-
Fejervarya limnocharis	10	48	16	-	-	-
Fejervarya nicobariensis	12	1	1	-	-	1
Hylarana baramica	-	-	-	-	-	1
Hylarana erythrea	21	5	-	1	-	-
Hylarana glandulosa	-	-	-	4	2	3
Hylarana labialis	-	-	-	39	5	5
Hylarana miopus	-	-	2	-	-	-
Hylarana picturata	-	-	-	5	1	-
Ingerophrynus parvus	-	3	-	-	-	6
Kaloula pulchra	-	23	21	-	-	-
Limnonectes blythii	-	-	1	15	6	-
Limnonectes laticeps	-	-	1	-	-	-
Limnonectes malesianus	-	-	-	8	6	-
Limnonectes plicatellus	-	-	-	-	-	1
Microhyla butleri	-	7	4	-	-	4
Microhyla heymonsi	1	18	35	-	-	-
Microhyla mantheyi	-	-	-	-	3	7
Microhyla superciliaris	-	-	-	-	-	4
Odorrana hosii	-	-	-	8	2	-
Phrynoides aspera	-	-	-	10	2	-
Polypedates leucomystax	1	3	17	-	-	-

Appendix G

Table G.1: Estimated coefficients of variables generated from non-truncated negative binomial linear and zero-truncated negative binomial linear model with standard error of coefficient for each model type. Variable host type (factor) was separated into abbreviated species names (refer to Appendix A for corresponding full names), with the Intercept corresponding to *Limnonectes blythii*.

Variable	**Non-truncated model (*SE*)**	**Zero-truncated model (*SE*)**
Intercept	-4.25 (*2.25*)	-5.10 (*0.48*)
Host(FLIM)	5.44 (*4.25*)	6.14 (*5.69*)
Host(HEY)	10.7 (*5.74*)	14.1 (*7.59*)
Host(MAN)	-11.4 (*5.29*)	-13.5 (*7.06*)
Host snout-vent	1.54 (*0.564*)	1.74 (*0.717*)
Host(FLIM):Snout-vent	-1.57 (*1.10*)	-1.78 (*1.47*)
Host(HEY):Snout-vent	-3.42 (*1.88*)	-4.59 (*2.50*)
Host(MAN):Snout-vent	4.06 (*1.67*)	4.78 (*2.23*)